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Investigating the Use and Identity of Traditional Herbal Remedies Amongst South Asian Communities Using Surveys and Biomolecular Techniques.

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Abstract

Herbal medicines (HMs) have been used to supplement, maintain, and treat health conditions, and have inspired the development of many Western pharmaceuticals. Migrant South Asian (SA) communities in the UK have brought with them their own traditional forms of medicine, yet little is known about their current use of HMs in the UK. Consuming HMs alongside conventional Western medicines could affect pharmacological treatment and lead to herb-drug interactions; hence, healthcare professionals (HCPs) should be aware of their patients' use of HMs. The import of HMs to the UK raises concerns over the quality, safety and regulation of HMs. Deoxyribonucleic acid (DNA) barcoding can be used to discriminate between different species, and identify contaminants and adulterants, thus can be used for the authentication of HMs.

The South Asian Traditional Medicines (SATMED) questionnaire explored the knowledge and use of HMs by diasporic SA communities in the UK. It uncovered a vast range of HMs which were used by participants, where ingredients were sourced from, the concurrent use of herbal and Western medicines, and how minor ailments were treated. An online survey designed to investigate UK based practitioners' views of HMs revealed that HCPs claimed to lack sufficient knowledge of HMs. HCPs said they needed more training on HMs to help them make better informed decisions. Tulsi (*Ocimum tenuiflorum* L.) was identified as a culturally and commercially valuable plant, which was used for molecular analysis. A variety of tulsi samples were collected for authentication: community samples from SA families in the UK, commercial samples, and referenced specimens. Both ITS and *trnH-psbA* regions were successfully used to distinguish between several *Ocimum* species, and identify a potential species substitution.

This research represents the first time that DNA based methods have been used to authenticate medicinal plants species used by migrant SA communities living in the UK. The results of this multi-disciplinary study provide a unique contribution to the evolving discipline of ethnopharmacology.

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Profile of Presentations

Presentations

Bi-annual presentations to Michael Heinrichs' Research Group - Update on PhD Project. Sukvinder Bhamra. The School of Pharmacy, University of London, September 2015.

Annual presentations at the Biomolecular Technology Group research seminars - Update on PhD Progress, Sukvinder Bhamra, De Montfort University, Leicester, June 2015.

Posters

DNA authentication of tulsi (*Ocimum tenuiflorum* L.) using the nuclear ribosomal internal transcribed spacer (ITS) and the chloroplast intergenic spacer *trnH-psbA.* Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. 63rd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), Budapest, August 2015.

The use of traditional herbal remedies by South Asian communities in the UK. Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. De Montfort University Post Graduate Research Conference, De Montfort University, Leicester, May 2015.

The secrets of South Asian traditional herbal remedies. Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. DMU poster competition, De Montfort University, Leicester, March 2015.

DNA authentication of tulsi; the cultural and medicinal value of *Ocimum* species among diasporic South Asian communities in the UK. Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. 62nd International Congress and Annual Meeting of the Society for Medicinal Plant and Natural Product Research (GA), Portugal, August 2014.

South Asian Traditional Herbal Medicines. Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. South Asian Health Foundation, Diabetes and Cardiovascular Disease in South Asians - The clinical conundrums, Birmingham, November 2013.

The Tulsi Project – collecting and sharing tulsi samples. Sukvinder Bhamra, Mark Johnson, Michael Heinrich, Caroline Howard, Adrian Slater. Ryton Gardens, Coventry, August 2013.

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Abbreviations

A	Adenine
ADRs	Adverse Drug Reactions
ANOVA	Analysis of Variance
AYUSH	Department Of Ayurveda, Yoga And Naturopathy, Unani, Siddha And Homeopathy
BG	Blood Glucose
BLAST	Basic Local Alignment Search Tool
BME	Black and Minority Ethnic
BNF	British National Formulary
BOLD	Barcode of Life Database
C	Cytosine
CAM	Complementary and Alternative Medicines
CBOL	The Consortium for the Barcode of Life
CCG	Clinical Commissioning Group
CITES	Convention on International Trade of Endangered Species
COX-1	Cytochrome C Oxidase
CWM	Conventional Western Medicines
CYP	Cytochrome
ddNTPs	Dideoxynucleotides triphosphates
DMU	De Montfort University
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleoside triphosphates
DTB	Drug and Therapeutics Bulletin
EIC	East Indian Company
EPS	European Protected Species
EU	European Union
FRLHT	Foundation for Revitalisation of Local Health Traditions
G	Guanine
GAfREC	Governance Arrangements for Research Ethics Committees
GDC	General Dental Council
GMC	General Medical Council
GPs	General Practitioners
GPhC	General Pharmaceutical Council
HCP	Healthcare Professional
HCPs	Healthcare Professionals
HM	Herbal Medicine
HMs	Herbal Medicines
HPLC	High Performance Liquid Chromatography
HPTLC	High Performance Thin Layer Chromatography
IBD	Inflammatory Bowel Disease
ILO	International Labour Office
INR	International Normalised Ratio
IRAS	Integrated Research Application System
ISCO	International Standard Classification of Occupations
ISM&H	Indian Systems of Medicines and Homoeopathy
ITS	Internal Transcribed Spacer
M	Mean

MA	Marketing Authorisation
MAOIs	Monoamine Oxidase Inhibitors
MHRA	Medicines and Healthcare Regulatory Agency
MUR	Medicines Use Review
n	Number of units in a subgroup
NHS	National Health Service
NHS R&D	National Health Service Research and Development
NMC	Nursing and Midwifery Council
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
O.	*Ocimum*
OTC	Over The Counter
P	Significance level
PAGB	Proprietary Association of Great Britain
PCR	Polymerase Chain Reaction
pdf	Portable Document Format
PIL	Participant Information Leaflet
PSNC	Pharmaceutical Services Negotiating Committee
RNA	Ribonucleic Acid
REC	Research Ethics Committee
SA	South Asian
SAs	South Asians
SATMED	South Asian Traditional Medicines
SEM	Scanning Electron Microscopy
SD	Standard Deviation
Sig	Significance
SJW	St John's Wort
SPSS	Statistical Package for the Social Sciences software
SSI	Site Specific Information
SSRIs	Selective Serotonin Re-uptake Inhibitors
T	Thymine
TCM	Traditional Chinese Medicine
THR	Traditional Herbal Medicine Registration
THM	Traditional Herbal Medicines
THMRS	Traditional Herbal Medicine Registration Scheme
TLC	Thin Layer Chromatography
TM	Traditional Medicine
UK	United Kingdom
UMC	Uppsala Monitoring Centre
US	United States
USA	United States of America
UV	Ultra Violet
WWW	World Wide Web
WHO	World Health Organisation
YCS	Yellow Card Scheme

1.0 Introduction

1.1 Introduction

''Plant derived medicines have been the first line of defence in maintaining health and combating diseases,'' (Samy et al., 2008). Nerkar (2007) claims that nature has provided a pharmacy which people have recognised and taken advantage of. Many Western pharmaceuticals have been developed from natural sources and the search for new drug leads has often taken scientists back to traditional herbal medicines (Harvey, 2008). Herbal medicines (HMs) are still commonly used in many cultures (Williamson, 2002); despite this, there is limited documented ethnopharmacological evidence of the use of HMs in the United Kingdom (UK).

The use of HMs is believed to be increasing as people use HMs to replace or complement Conventional Western Medicines (CWM), and seek natural ways to supplement a healthy lifestyle (Newall et al., 1996; Mintel, 2009). There is a common misconception that HMs are natural and therefore safe and free from harmful effects (Vickers, 2000; Ipsos MORI, 2008); however, there remains a lack of clinical evidence to support the safety and efficacy of HMs. There could be risks associated with the simultaneous consumption of HMs with CWM such as herb-drug interactions and adverse effects. To ensure patients are getting the best pharmaceutical care, Healthcare Professionals (HCPs) need to recognise that their patients may be using HMs in addition to CWM (Shaw et al., 2012). HCPs need to have sufficient training and knowledge of HMs to help patients make informed decisions about their health (Newall et al., 1996).

Sandhu and Heinrich (2005) note that, ''*in the past decades a significant immigration from outside Europe has influenced European societies in a multitude of ways''* and observe that immigrants have brought their own culture, culinary skills, and traditional herbal remedies with them. The ethnic diversity in the UK means there is a great wealth of knowledge of HMs and variety of HMs which may be used. The increasing migration of people from the Indian subcontinent countries of India, Pakistan and Bangladesh (South Asia) to the UK has created large South Asian (SA) populations in the UK (Khunti et al., 2009). Results from the 2001 census revealed that SA ethnic minorities formed 3.9% of the UK population; this increased to over 5.3% in the 2011 census (Office for National Statistics, 2012). The SA ethnic group is itself very diverse and comprised of different cultures and religions all with their own interpretations of traditional herbal medicine; and little is known about the current use of HMs by SA populations in the UK. This presents a unique opportunity to explore the use of HMs by SA ethnic populations.

The import and increased use of HMs in the UK has introduced concerns over the quality, safety, and regulation of HMs (Vassou et al., 2015); hence, reliable and efficient methods of identification are needed. DNA-based techniques have been developed to authenticate medicinal plants used in HMs. These methods can be used to identify the plant species and any potential substitutes or adulterants; a relatively new technique which will be explored further in this research.

This chapter will give an overview to the rationale for this study; detailing the aims, objectives, and background information to this research. A brief overview of the structure of the thesis will explain how the project evolved.

1.2 Research Aims

The aim of this research is to explore the knowledge and use of traditional herbal remedies amongst South Asian communities and healthcare professionals in the UK; followed by the collection of specific medicinal plants of interest, which can be genetically tested to confirm their identity, using DNA-based techniques.

1.3 Objectives

1. Explore the knowledge and use of traditional herbal remedies amongst South Asian diasporic communities, in the UK; describe where the knowledge originates from.
2. Obtain insight into UK based healthcare professionals' knowledge and opinions of herbal medicines.
3. Use DNA barcoding techniques for the identification of herbal medicines and medicinal plants used in South Asian communities.

1.4 Background to This Research

In order to gain perspective on the origins and current use of HMs some background information is provided in this section. It explores the origins of traditional HMs, HMs as drug leads, the use of HMs, some potential problems associated with HMs (i.e. contamination and adulteration), and the regulation of HMs in India and Europe.

1.4.1 The Origins of Traditional Herbal Medicines

Human beings have evolved alongside plants, which have been used as food and medicine for millennia (Chevallier, 2007). People learnt by trial and error which plants, seeds, berries and fruits were good to eat; as a consequence they became aware of the potential health benefits. Hatfield (2007) suggests the precise origins of HMs are unclear, but it is likely to have occurred as a by-product of the use of plants in other ways; for example, chewing the leaves of a particular plant may have caused the mouth to feel anaesthetised, leading to the plant being used for toothache, or a plant rubbed on the skin to wipe a wound may have helped it heal establishing its use in wound healing. The knowledge of which plants are medicinally beneficial comes from their use as food and traditional cultural backgrounds (Chevallier, 2007). Folk practice of medicine, using plants to treat and prevent diseases, began as an oral tradition and has been passed down generations, long before written records were created (Hatfield, 2007).

Around the world there are many different cultures which have created their own system of traditional medicine to diagnose, prevent and treat illnesses (Wachtel-Galor and Benzie, 2011). Ayurveda, a traditional Indian medical system, is believed to be more than five-thousand years old, and is said to be one of the oldest medical systems known; it is a holistic approach to managing health and preventing disease (Williamson, 2002; Sharma et al., 2007). Ancient Chinese traditions dating back to the Stone Age claim that man gradually learnt to recognise the properties of plants, and then used them as medicine (Hong, 2004). Egyptian papyrus scripts as early as 3000 BC found preserved, contained hundreds of hieroglyphics detailing specific medical conditions and herbal treatments (Ehrlich, 2011). Pedanius Dioscordes the famous Greek physician published the first European herbal pharmacopoeia, around 65 AD, called *De Materia Medica*. Dioscordes organised all the plants in a systematic manner, grouping plants with similar pharmacological actions together (Isely, 1994). More recently Saslis-Lagoudakis et al. (2011) demonstrated the applications of phylogeny in mapping the pharmacological properties of ethnomedicinal plants and bioscreening. Their phylogenetic study comparing cross cultural uses of ethnomedicinal plants uncovered that similar traditional uses were found in different areas where the related species were available. Throughout history and across the globe plants have been used as the primary source of medicine (Hatfield, 2007).

According to the World Health Organisation (WHO, 2003), *"Traditional medicine refers to health practices, approaches, knowledge and beliefs incorporating plant, animal and mineral based medicines, spiritual therapies, manual techniques and exercises, applied singularly or in combination to treat, diagnose and prevent illnesses or maintain well-*

being". WHO (2008) clarifies that the terms complementary/ alternative/ non-conventional medicines are used interchangeably when referring to traditional medicines, and the terms allopathic/ conventional are used to refer to Western medicines. Herbal medicines form an integral part of Complementary and Alternative Medicines (CAM), which is a broad term used to describe hundreds of therapies including: massage, nourishment, exercise, and HMs (Kayne, 2002).

For the purpose of this study the terms herbal medicine(s), traditional herbal medicine(s), herbal remedy/remedies, and traditional herbal remedy/remedies will be used interchangeably.

1.4.2 Herbal Medicines as Drug Leads

Approximately 25% of drugs prescribed worldwide are derived from plants (Sahoo et al., 2010); for example, aspirin based on the chemicals found in willow bark (*Salix* spp.) is used for pain relief and anti-platelet effects, and digoxin from the foxglove plant (*Digitalis lanata* Ehrh.) is used for cardiac disease (Hartmann, 2007; Li and Vederas, 2009). Metformin, an oral anti-diabetic drug, often the first line treatment for type 2 diabetics is also derived from plants. It originates from French lilac or Goat's rue (*Galega officinalis* L.) a plant used in traditional medicine for centuries, with the first references documented in 1500 BC by Egyptian physicians (Witters, 2001). The use of papaverine from the opium poppy (*Papaver somniferum* L.) as a vasodilator led to the development of verapamil for hypertension. More commonly known are the opioid analgesics codeine and morphine for pain relief which are also derivatives of the opium poppy (Cragg and Newman, 2013). In 1820 French pharmacists Pierre Joseph Pelletier and Joseph Caventou isolated and purified quinine from the bark of the cinchona tree (*Cinchona officinalis* L.) which was used to treat malaria. The traditional use of quinine for treating fever and infectious disease dates back to the 1600s (Achan et al., 2011). Since then quinine has formed the basis of the development of other anti-malarial drugs such as cholorquine and mefloquine. More than 60% of cancer drugs on the market or being researched are based on natural products (Wachtel-Galor and Benzie, 2011); including the vinca alkaloids vinblastine and vincristine isolated from the Madagascar periwinkle (*Catharanthus roseus* L.) (Cragg and Newman, 2013).

Over the past hundred years developments in technology have revolutionised health care across most parts of the world, where once HMs were the only source of medication (Wachtel-Galor and Benzie, 2011). Plants have the ability to synthesise a variety of chemical compounds which can be consumed by humans for maintaining health, treatment and prevention of disease. Advances in chemistry have made it possible to extract, isolate, and

synthesise compounds from natural products. This has been a contributory factor to the decline in use of plants for primary care and a rise in the use of synthetic drugs (Sahoo et al., 2010; Dias et al., 2012). Nevertheless, the search for potential therapeutic agents continues, with nearly half of the small molecules approved in this decade having originated from natural products (Kong et al., 2009; Pandikumar et al., 2011).

As the process of drug discovery is becoming more expensive, reverting back to traditional remedies may provide a cheaper alternative for pharmaceutical companies (Patwardhan and Khambholja, 2011). Patwardhan and Mashelkar (2009) claim that as natural products have been used for a long time, there is more information available on tolerance and safety compared with the development of novel synthetic drugs which have never been used before. However, most of this traditional knowledge is still not documented or supported by clinical trials. Patwardhan and Mashelkar (2009) propose a "reverse pharmacology approach" whereby products traditionally known to have clinical effects are scientifically tested; they go on to say, *"The novelty of this approach is the combination of living traditional knowledge such as Ayurveda and the application of modern technology and processes to provide better and safer leads."*

Natural resources which have been traditionally used by indigenous people have been commercially exploited by corporations; this is known as biopiracy (Robinson, 2010). Several high profile cases of biopiracy including basmati, neem and turmeric have led to the development of guidelines to protect indigenous knowledge and resources (Robinson, 2010). In 1995 two scientists in America got a patent for the use of turmeric in wound healing. The patent was revoked in 1997 after a complaint was filed by India's Council of Scientific and Industrial Research, who highlighted that turmeric had traditionally been used in wound healing for thousands of years (Nobel, 2014). Since then a database (Traditional Knowledge Digital Library) to document traditional knowledge of medicinal plants and ancient formulations has been developed by the Indian Council of Scientific and Industrial Research, Ministry of Science and Technology, and the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) to protect traditional knowledge from being exploited by biopiracy (Gupta, 2011). Patenting laws to protect indigenous species have been introduced in several countries including China, India, Peru and the Philippines (Robinson, 2010).

Scientists are continuously exploring the benefits natural products offer in anticipation of discovering new drug leads and developing novel drugs. Ayyanar and Ignacimuthu (2011) suggest further ethnopharmacological studies are required for discovering potential new drugs.

1.4.3 The Use of Herbal Medicines

Hatfield (2007) declares that the use of medicinal plants is part of our heritage, but fears this valuable knowledge is on the brink of extinction. Despite this, it is estimated that in some developing countries more than 80% of the population depend on HMs for primary health care (WHO, 2008). Up to 90% of the population in Africa and 70% in India depend on traditional medicine to meet their healthcare needs (WHO, 2005). This could be due to poverty, a lack of access to or knowledge of modern medicine (Ayyanar and Ignacimuthu, 2011). In India the HMs market is immense; 960 medicinal plants have been widely incorporated into the HMs industry, of which 178 have been identified as high volume due to popularity and demand (Sahoo et al., 2010). However, the use of HMs is not limited to developing countries; over the past few decades interest in HMs in developed countries has been rising (Ernst and White, 2000; Pharmaceutical Press, 2013). In Europe, Germany and France are leading in over-the-counter (OTC) sales of HMs, which are readily available even in conventional pharmacies (Wachtel-Galor and Benzie, 2011). The WHO (2003) factsheet on traditional medicines claims that in Germany 90% of the population have used a natural remedy at some time in their life. The HMs market value is steadily growing; the annual expenditure on CAM in Australia, Canada and the UK, was estimated to be worth US$ 80 million, US$ 2400 million, and US$ 2300 million respectively (WHO, 2002a). In the United States of America (USA) the use of CAM has been steadily increasing since the 1950s (Kessler et al., 2001); in 2011 the annual retail sales value of HMs in the USA was estimated to be over US$5.2 billion (Blumenthal, 2012).

According to market research conducted by Mintel (2009), complementary therapies including Ayurvedic medicines, Traditional Chinese Medicine (TCM) and homeopathic remedies are becoming more popular in the UK. The report titled *'Complementary medicines- UK, December 2009,'* (Mintel, 2009) identified that the number of people trying complementary medicines was increasing, with over twelve-million people reported to have used them in the UK in a single year. In 2009 the UK's complementary medicines market was worth £213 million, and a 20% growth was predicted for the next two years (Mintel, 2009). A survey to explore the publics' perceptions of HMs in the UK in 2008 was commissioned by the Medicines and Healthcare products Regulatory Agency (MHRA). The results uncovered that 35% of the population surveyed claimed to have used HMs (Ipsos MORI, 2008). In 2013 a review of ten surveys based on UK patients and HMs consumers indicated that on average 37% of the population used HMs (Ministry of Foreign Affairs, 2014). The UK HMs market is relatively strong, especially with a diverse ethnic population that is interested in non-Western alternatives (Ministry of Foreign Affairs, 2014). Wachtel-Galor and Benzie (2011) suggest some of the reasons people use HMs include: cost (i.e.

more affordable than conventional therapies), part of traditional background, HMs perceived to have less adverse effects than chemical alternatives, to replace or supplement CWM, and the notion of natural medicines being safer.

1.4.4 Potential Problems with Herbal Medicines

1.4.4.1 Destruction of Natural Resources

There may be important implications for the sustainability of natural resources; as the increasing demand for HMs could lead to overharvesting of endangered species and destruction of natural resources (Wachtel-Galor and Benzie, 2011; Booker et al., 2012). Alam and Belt (2009) claim, *"Medicinal plant species are depleting at a rapid pace due to large-scale, unsustainable collection from their natural habitats."* According to one estimate over four thousand economically important species are threatened with extinction due to commercial over-harvesting to meet manufacturers demands (Schippmann et al., 2002).

There are approximately 29,000 plant species protected by the Convention on International Trade of Endangered Species (CITES) (Hollingsworth et al., 2011); for instance, American Ginseng (*Panax quinquefolius L.*) is native to the USA and can only be legally harvested in nineteen states with special restrictions controlling the growth, harvesting, sales, and export (US Fish and Wildlife Services International Affairs, 2015). In Europe there are several plant species which are listed as European Protected Species (EPS) under the European Habitats Directive, including shore dock (*Rumex rupestris* Le Gall), traditionally used as an astringent, and marsh saxifrage (*Saxifraga hirculus* L.) (Department for Environment, Food and Rural Affairs, 2014).

The conservation of medicinal plants is of paramount importance as they remain an important source for natural ingredients for HMs, and there are potentially many more active compounds yet to be screened (Alam and Belt, 2009).

1.4.4.2 Standardisation of Herbal Medicines

Due to natural variance in the chemical composition of plants it can be difficult to standardise HMs (Wachtel-Galor and Benzie, 2011). Factors such as the climate, soil, genotype, parts of the plants used, time the plants are harvested, storage, drying, and manufacturing process can all affect the chemical composition of medicinal plants (Shaw et al., 2012); making it difficult to ensure the reproducibility of HMs (Pharmaceutical Press, 2013). Manufacturers of HMs are required to standardise and quantify the active constituents

in formulations according to Pharmacopoeia standards (Waimer and Schwabe, 2011); however, if the raw plant material is consumed or unregulated formulations are used it is difficult to ascertain the chemical composition of the herbal remedy.

HMs used in the UK come from all over the world, from a range of traditional medical systems including TCM and Ayurveda; therefore, problems with nomenclature and standardisation of names can occur (Shaw et al., 2012). In the USA and Europe the Latin binomials names are used as the official way of referring to plants; however, traditional names may be different and could lead to confusion when selecting the plant species. For example, in the Philippines ginseng was popular for its use as a male aphrodisiac but this "ginseng" was not the same as the ginseng which belongs to the genus *Panax*; instead it was *Jatropha podagrica* Hook. commonly known as the Buddha belly plant (Begg and Gaskin, 1994; Bareja, 2010). It remains unclear how the Buddha belly plant was used instead of ginseng; it may have occurred as the plants looked similar, or due to the commercial value of ginseng whereby the Buddha belly plant was a cheaper alternative. When the traditional and binomials names of species are different it increases the risk of species substitution and adulteration, which may be accidental or deliberate. The Royal Botanical Gardens, Kew, has created a database called the 'Medicinal Plants Names Service' which is a resource for searching information about medicinal plant names. As different names may be used for the same plant (i.e. in different countries, communities, and languages) and even the Latin binomials themselves change; this database has collated all the relevant published information about medicinal plant names enabling more accurate communication and identification of medicinal plants (Medicinal Plants Names Service, 2015).

1.4.4.3 Species Substitution and Adulteration

Reports of commercially valuable species being substituted for cheaper alternatives have been documented (Vassou et al., 2015). Ginseng (*Panax ginseng* C.A.Mey) has numerous health benefits; it has been used for fatigue, enhancement of mental and physical performance, and as a male stimulant (Chevailler, 2007). The rising demand for ginseng has led to an increased commercial value and a decline in natural resources. Dharmananda (2002) identified several cheaper and more abundant species which were used as substitutes for ginseng in TCM including: *Codonopsis pilosula* Nannf. (also known as dang shen or poor mans' ginseng) which is unrelated to ginseng but believed to have a similar action, *Rumex madaio* Makino and *Talinum paniculatum* (Jacq.) Gaertn. which look similar to ginseng roots.

A Chinese slimming aid in which *Stephania tetrandra* S.Moore had been substituted by *Aristolochia fangchi* Y.C.Wu ex L.D.Chow & S.M.Hwang caused renal damage in over one hundred patients in Belgium (Vanherweghem, 1998). S*tephania tetrandra* is traditionally referred to as *'Fangchi'* or *'Fangji'*, while *Aristolochia fangchi* is referred to as *'Mu Fangchi'* or *'Mu Fangji'*; the similar traditional names may explain how the confusion of the species occurred (Sim et al., 2013). The chemical composition of the two species is very different; aristolochic acids found in *Aristolochia* species have been known to be nephrotoxic, mutagenic, and carcinogenic. The use of *Aristolochia* species has since been restricted and prohibited in several countries including Australia, Canada, Germany, and the UK (Vanherweghem, 1998; Michl et al., 2014).

The World Health Organisation (WHO) has emphasised the need to control the quality of HMs by using modern control techniques and standards (WHO, 1998). The application of molecular techniques in identifying substitute and adulterant species in HMs has been widely investigated; hence, DNA-based techniques have been used to accurately recognise plant species in HMs (Howard, 2010). Vassou et al. (2015) highlight the problems of species substitution and adulteration in their study of Indian country mallow also known as *'Bala'* in Ayurveda (*Sida cordifolia* L.). They bought 25 market samples of raw material from thirteen states in India. They used DNA barcoding techniques to identify the species of the samples collected, and found that none of the samples were *Sida cordifolia*; instead, 76% were identified as other *Sida* species and the remaining 24% were from other genera. Several of the samples were identified as *Sida acuta* Burm.f. which has six times more ephedrine in comparison to *Sida cordifolia*; this may be associated with adverse effects. The research by Vassou et al. (2015) signifies the importance of species authentication of HMs and the applications of DNA barcoding for this purpose.

1.4.4.4 Contamination of Herbal Medicines

Substitution of ingredients or species, and contamination with toxic substances may be a particular concern especially when HMs are not regulated (Pharmaceutical Press, 2013). On several occasions Ayurvedic formulations have been found to contain heavy metals such as iron, mercury, and arsenic which can release toxic compounds in the body altering normal metabolic pathways, and can cause systemic toxicity (Ernst, 1998; Egbuna and Bose, 2005). Saper et al. (2008) found nearly two hundred Ayurvedic preparations bought over the internet had detectable levels of heavy metals which were not stated on the label. A British poison information centre, in the West Midlands, identified five cases of heavy metal contamination within seven years; the products contained lead, zinc, mercury, arsenic,

aluminium, and tin. The individuals who had consumed the contaminated HMs had concentrations of heavy metals 2-10 times the upper limit of normal physiological values, resulting in harmful effects and morbidities (Bayly et al., 1995).

Contamination with undeclared prescription medicines, mefenamic acid and diazepam, was identified in some Chinese HMs; they caused severe adverse effects including gastrointestinal bleeding and renal failure (Gertner et al., 1994). HMs contaminated with microbes due to poor manufacturing, harvesting, and storage conditions have also been documented (Zhang et al., 2012). This identifies that stringent controls need to be reinforced over the manufacture, sales and supply of high quality and safe HMs.

1.4.4.5 Interactions between Herbal and Conventional Medicines

Herbal medicines have been used long before written records were created; despite this there is still limited clinical evidence to support the safety and efficacy of most HMs (Ernst, 1998). The incidence of herb-drug interactions are still not fully known, and there is no reliable body of information to assess the potential problems (Wachtel-Galor and Benzie, 2011; Williamson et al., 2013).

An interaction is believed to occur when the effects of a drug are changed by the presence of another substance (Baxter, 2008). The effects of the drug may be mimicked or opposed by the herbal medicine; which in turn may alter the efficacy of the drug. For example, St John's Wort (*Hypericum perforatum* L.) is known to be an enzyme inducer, affecting the Cytochrome P450 enzymes; it has been associated with reducing levels of ciclosporin leading to transplant rejection as the efficacy of the drug was reduced (Graham et al., 2008; Williamson et al., 2013). There is an increased risk of toxicity if a drug's metabolism and clearance is inhibited, especially for drugs with a narrow therapeutic range such as digoxin, lithium, or theophylline (Colalto, 2010). Due to the lack of controlled research it is difficult to ascertain the prevalence of herb-drug interactions. This has been explored further in Chapter 3, section 3.4.4.1 (p91).

1.4.4.6 Adverse Effects of Herbal Medicines

There may be many side-effects associated with HMs which are not acknowledged; Ravindran et al. (2009) believe, *''traditional medicines are generally free of the deleterious side effects.''* In contrary Balaji (2010) expresses concerns of toxicity in using raw plant material / extracts by stating, *''laboratory findings suggest that turmeric extracts can be*

toxic to the liver when taken in high doses, or for a prolonged period of time.'' There are several well-known Adverse Drug Reactions (ADRs) caused by the consumption of HMs including: burning sensation and skin irritation caused by chillies (*Capsicum* spp.) or *Aloe vera* L., allergic reactions to celery or nuts, blood disorders associated with garlic, and aristolochic acid induced nephrotoxicity (Chevallier, 2007; Michl et al., 2014).

The Uppsala Monitoring Centre (UMC) collates information on ADRs from over one hundred countries and in 2010 it found 12, 679 reports of ADRs linked to HMs (UMC, 2011). Adverse effects linked to HMs reported included: anaphylaxis, anxiety, dermatitis, chest pain, toxicity and death; due to heavy metal and microbial contamination, pesticide residues, and also the wrong species of plant used in preparation of the medicine (Sahoo et al., 2010). In the UK, the MHRA established the 'Yellow Card Scheme' (YCS) to monitor the safety of healthcare products including HMs; it was designed to enable HCPs and the public to report side-effects and ADRs caused by drugs and HMs (MHRA, 2014a). The reporting of herbal ADRs remains low, just 51 reports of herbal ADRs were received by the MHRA in 2014 (MHRA, 2014b; MHRA, 2014c).

1.4.5 Regulation of Herbal Medicines

In response to some of the quality issues identified with HMs, the Department of Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homoeopathy (AYUSH) was established by the Department of Indian Systems of Medicines and Homoeopathy (ISM&H) in 2003 to regulate HMs in India. AYUSH proposed to regulate Ayurvedic formulations made in India that are often distributed worldwide, by focusing on research, quality control, and education relating to HMs. The Indian government declared that traditional herbal remedies needed scientific validation to promote national and international use of HMs which have been used for centuries (Sharma, 2000). The responsibility for ensuring the safety and standards of Ayurvedic preparations lies with AYUSH. The labelling requirements are clearly stated in AYUSH guidelines (Section III, Annex A). The scope of the AYUSH regulation covers, *"the product requirements, good manufacturing practices, requirements for competence of personnel and testing of AYUSH products included in the active pharmaceutical ingredient,"* (AYUSH, n.d.). An investigation by Saper et al. (2008) found nearly two hundred Ayurvedic preparations bought over the internet had detectable levels of heavy metals, which were not stated on the label. This highlights that products failing to comply with the guidelines are still readily available. There are concerns about the quality and safety of HMs bought online as it is difficult to regulate what is being sold via the internet (The Pharmaceutical Journal, 2002).

In Europe, the Traditional Herbal Medicinal Products Directive (THMPD) was passed in 2004 by the European Parliament and Council of Europe to set out guidelines to regulate the use of HMs (Directive 2004/24/EC). Previously HMs could be marketed without an official Marketing Authorisation (MA), as long as the unlicensed products were of high quality and safe for consumption, as decided by each country on a national level. The 2004/24/EC directive led to the development of Traditional Herbal Medicine Registration Scheme (THMRS) in the UK, which meant over-the-counter HMs which made a medicinal claim, would require a Traditional Herbal Medicine Registration (THR) or a MA, by May 2011. The legislation required herbal medicinal products to have at least 30 years of evidence of their traditional use in the EU; or 15 years within the EU and 15 years elsewhere for products from other countries (European Parliament, 2004). In addition, it required safety data to be provided and the manufacturer was obliged to guarantee the quality of the product with regards to Good Manufacturing Process (GMP) and European Pharmacopoeia standards. Manufacturers were also expected to ensure pharmacovigilance procedures were in place to monitor ADRs (Pharmaceutical Press, 2013). The idea was to regulate high quality and safe HMs, in addition to giving consumers the confidence in the products they were using. As of the 30th April 2011 no unlicensed herbal medicinal product could be sold in the EU unless they held a product license. Products on the market before this date were given a three year period to sell through, until 30th April 2014.

The Directive 2004/24/EC defines herbal medicinal products as, "*Any medicinal product, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations."*

It also defines:

- Herbal substances as, *"All mainly whole, fragmented or cut plants, plant parts, algae, fungi, lichen in an unprocessed, usually dried, form, but sometimes fresh. Certain exudates that have not been subjected to a specific treatment are also considered to be herbal substances. Herbal substances are precisely defined by the plant part used and the botanical name according to the binomial system (genus, species, variety and author),"*

- Herbal preparations as, *"Preparations obtained by subjecting herbal substances to treatments such as extraction, distillation, expression, fractionation, purification, concentration or fermentation. These include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices and processed exudates."*

1.5 Overview of the Research Project

The initial purpose of this research was to explore the use of HMs amongst diasporic SA communities in the UK, followed by the identification of plant species in HMs using molecular techniques (Appendix 1). As DNA-based identification techniques are a relatively new way of authenticating plant species, the research team was interested in studying this phenomenon further; with the prospect of developing a specific, fast, and reliable identification tool for a selected plant species.

The first phase of this study was called the South Asian Traditional Medicines (SATMED) Project (Chapter 2); whereby, the primary aim of investigating the current use of HMs by SA communities in the UK was explored. During the development of the SATMED project a questionnaire was designed to explore whether participants were using HMs, what they were using, where ingredients were sourced from, and where their knowledge of HMs originated. One of the key themes involved exploring whether or not participants shared the information about their use of HMs with their Western HCPs. After exploring a 'patients' perspective of HMs the researcher was intrigued to understand what HCPs thought of HMs. As there is very limited research which has looked at UK HCPs knowledge and views of HMs, it was anticipated that the results would provide an original contribution to the gap in the existing literature. An online survey to explore HCPs personal and professional experiences with HMs was created and distributed using social media (Chapter 3).

From the results of the SATMED questionnaire a medicinal plant or herbal remedy which could be used for molecular analysis was identified. Tulsi (*Ocimum tenuiflorum* L.) was selected as the medicinal plant for a case study of the application of DNA analysis for confirmation of genetic identity, as tulsi was identified as a culturally and commercially valuable plant during the research period (Chapter 4). The cultural, religious and medicinal uses of tulsi were investigated during interviews with participants. Tulsi samples from participants, commercial sources, and authenticated specimen were collected for DNA analysis. This phase of the research was called the 'Tulsi Project'.

All three aspects of the research overlap as the concepts are a continuation from each other (Figure 1-1). The Venn diagram in Figure 1-1 represents some of the key ideas explored within each section and some of the common themes which emerged through this research. Ethical approval from the De Montfort University Ethics Committee for each phase of the research was obtained separately (Appendix 1 & 2); further details can be found in each chapter (Chapter 2, 3, and 4).

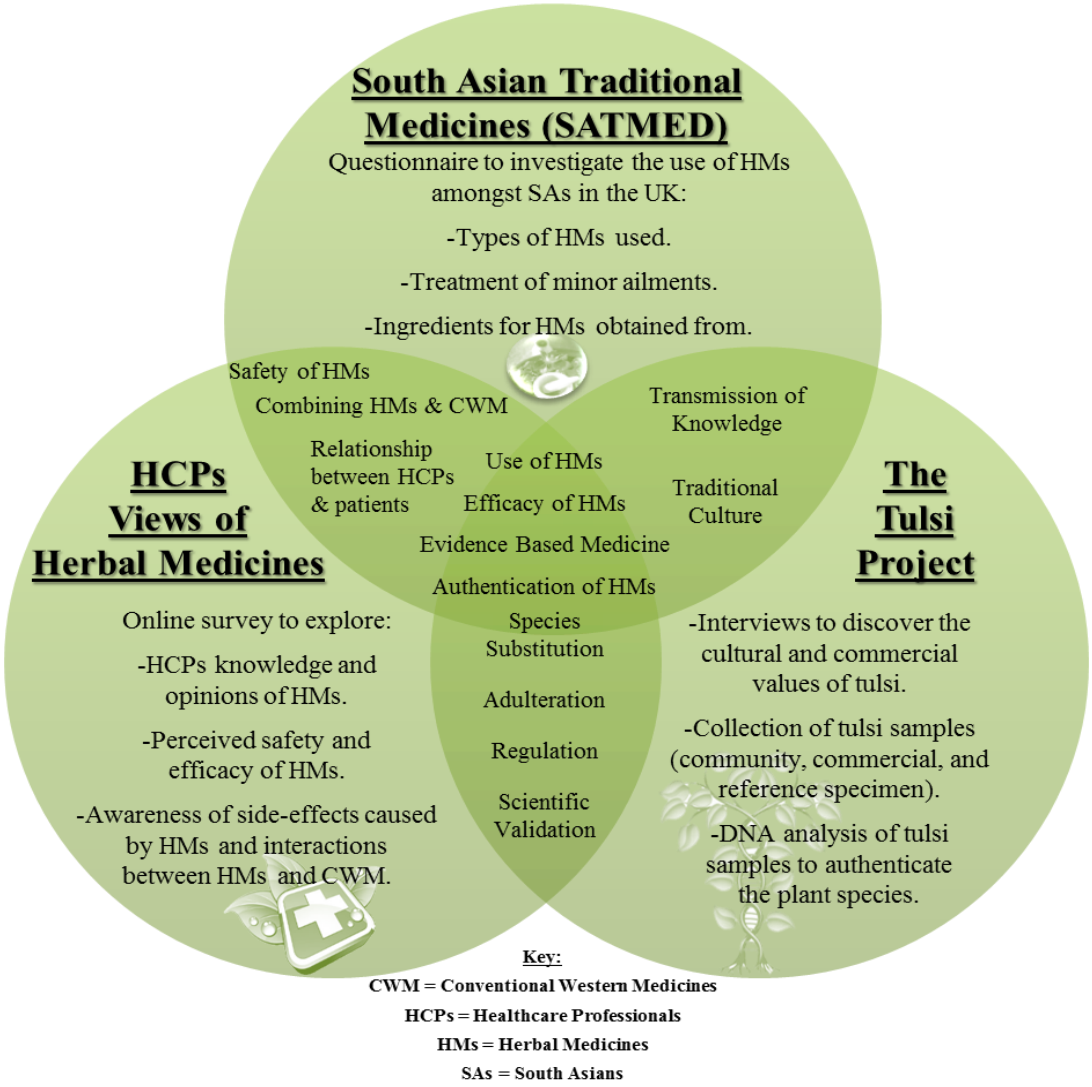


Figure 1-1 Venn Diagram to Illustrate the Integration of the Three Phases of the Research

The Venn diagram shows how the SATMED Project, HCPs survey and the Tulsi Project interlink to explore the aims and objectives of this study.

A detailed introduction in each chapter explains the aims, objectives, and rationale for the section. As the methodology was tailored to each phase it is detailed within the specific chapter; followed by a detailed analysis of the results and a discussion. The final discussion chapter (Chapter 5) summarises the key findings of this research and illustrates how the three strands of this investigation integrate and support each other; furthermore, it highlights the implications of this research and further work which could be conducted.

2.0 South Asian Traditional Medicines (SATMED) Project

2.1 Introduction

The migration of South Asians (SAs) to the United Kingdom (UK) has created an ethnically diverse population; with SAs forming 5.3% of the total UK population as uncovered by the 2011 Census (Office for National Statistics, 2012). With the migration of people to the UK they have brought with them their own herbal remedies which they may still be using; however, there is little documented evidence to support the current use of herbal medicines (HMs) by SAs in the UK (Etkin and Elisabetsky, 2005; Sandhu and Heinrich, 2005; Pieroni and Torry, 2007). This raises several interesting questions such as where the knowledge originates from, how it is transmitted through generations, where ingredients for traditional remedies are obtained from, and if HMs are used in conjunction with conventional Western medicines (CWM) are Western healthcare professionals (HCPs) aware. The South Asian Traditional Medicines (SATMED) project was designed to be an exploratory survey to establish the current use of HMs by South Asian (SA) communities in the UK. This chapter will summarise the aim and objectives of this research, followed by some background information to support the need to conduct this investigation. The methodology will describe the research methods used for the SATMED project and the results section will give a detailed analysis of the findings of this study. The terms herbal remedies, traditional herbal medicines (THM), and herbal medicines (HMs) will be used interchangeably throughout this research.

2.2 Aim of the SATMED Project

The aim of the SATMED project is to explore the origins, use, and transmission of knowledge of traditional herbal medicines used amongst South Asian diasporic communities in the UK.

2.3 Objectives of the SATMED Project

1. Explore the knowledge and use of traditional herbal remedies amongst South Asian diasporic communities; describe where the knowledge originates from and how it is transmitted.
2. Investigate where ingredients for herbal medicines are sourced from.

3. Obtain insight into the relationship between South Asian people who use herbal medicines and their healthcare professionals.
4. Summarise the types of herbal medicines and plants used within South Asian communities; to establish which plant species would be suitable candidates for DNA authentication.

2.4 Background Research

In order to establish why focusing on SA populations for this research is important, the background of how migration to the UK first started will be considered. Then the types of HMs and traditional medical systems used by SAs will be established to determine the origins of HMs, and evidence to support the use of HMs will be explored.

2.4.1 South Asians in the UK

2.4.1.1 The Formation of South Asia

The first British men went to India as traders as part of the East Indian Company (EIC) in search of spices, silk, cotton, ivory and tea. The Indian subcontinent was rich in natural resources which Europeans wanted (Kaul, 2011). The EIC began to rule areas of India and developed its own armies. After almost a century of oppressive control in India by the EIC the Indians rebelled and the British Government rule was established in 1858 (Lambert, 2014). The two hundred years of British rule in India came to an end in 1947 after which India, which was once bigger than Europe, was partitioned into independent states. The main body was left as India while the eastern and western wings were called Pakistan (Figure 2-1). In 1971 East Pakistan was renamed as Bangladesh. After this the three states and Sri Lanka collectively formed South Asia, and its people were referred to as South Asians (Healy and Aslam, 1989; Clarke et al., 1990; Brown, 2006).

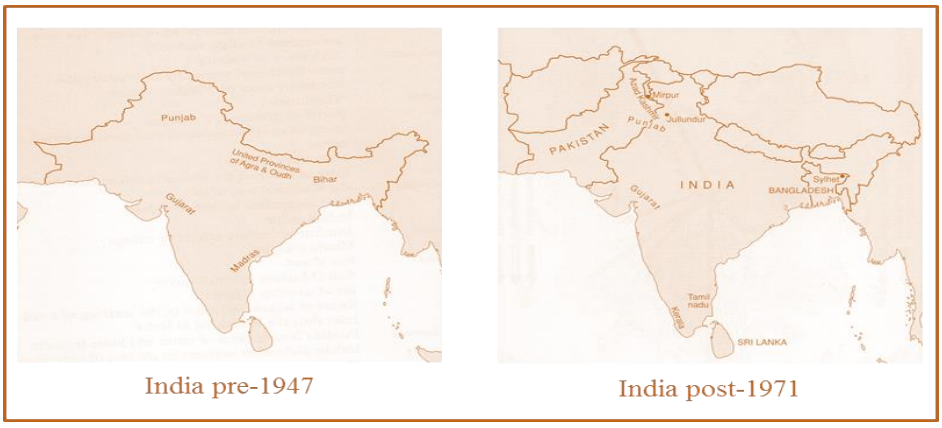


Figure 2-1 Map of India Pre and Post Partitioning (adapted from Brown, 2006)

2.4.1.2 Migration of South Asians to the UK

Migration from South Asia started early in the 19th century when SAs first moved to countries such as Mauritius, British Guiana, and Trinidad (Clarke et al., 1990). By the late 20th century SAs had become widely spread across Europe, Africa, Asia, America, and the Middle East (Brown, 2006). Reasons for migration may have been forced (e.g. contracts of indenture or refugees), or free willed - the migration of traders, entrepreneurs, labourers, and professionals. Service in the British army took men from India across the world and as transport links improved, migration became easier so people emigrated to seek new opportunities.

During the partitioning of India in 1947 there was large-scale immigration to the UK from India and Pakistan due to the violence and unrest. This first mass migration can be broadly divided into religious groups as Sikhs from India and Muslims from East and West Pakistan. These immigrants clustered in inner city areas of London, Manchester and the West Midlands, working in factories, with little or no education and qualifications (Hanif and Karamat, 2009). The influx continued until restrictions were set by the Commonwealth Immigrants Act of 1962 (Healy and Aslam, 1989).

During the British reign in India between 1858 and 1947 many Indians were educated or trained and taken to other countries to work in British colonies. Indians were used to work in clerical, manual labour and farming roles. Thirty two thousand Indians were taken to Southeast Africa under indentured labour contracts to build the Uganda railway networks (Kasozi et al., 1994). When East African Governments created polices to prevent British passport holders from working, many Asians who had settled there were left unemployed (Healy and Aslam, 1989). On the 4th August 1972 the president of Uganda, Idi Amin, declared that all Asians would be expelled from Uganda within ninety days. At the time of expulsion there were seventy five thousand Asians living in Uganda. Many held British passports so were able to immigrate to the UK (Kasozi et al., 1994). These immigrants were mostly of Gujarati origin and they settled around Leicester and London; they were better educated and many had successful businesses (Hanif and Karamat, 2009). According to Healy and Aslam (1989) the main types of Asians found in the UK can be categorised as Punjabi, Gujarati, Pakistani, Bangladeshi, and those East African Asians usually of Punjabi or Gujarati origin from Kenya, Uganda, Tanzania and to a lesser extent Malawi or Zambia. The SA population in the UK can be described as being heterogeneous due to the variety of religions, cultures, languages, social and economic backgrounds (Gill and Lloyd, 2009; Hanif and Karamat, 2009).

Diaspora comes from the Greek word (διασπορά) which means to disperse, scatter or spread. Brown (2006) uses the word diaspora to refer to groups of people with a common ethnicity who have left their original homeland but retained a cultural kinship. Although South Asia includes India, Pakistan, Bangladesh, Nepal and Sri Lanka, when referring to SA diasporic communities in this study it will focus on Indian, Pakistani and Bangladeshi communities as agreed by most official and research bodies (Khunti et al., 2009; Office for National Statistics, 2012).

2.4.1.3 UK Census Statistics

The 1991 Census was the first to account for ethnic minorities in the UK (Ballard, 2004). The SA ethnic minority encompassed Indians, Pakistanis and Bangladeshis. Of the total population 5.5% was identified as ethnic minority of which half was of SA origin (Brown, 2006). The total SA population in the UK has continued to rise from 3.9% in 2001 to 5.3% in 2011; Figure 2-2 illustrates the changes in SA ethnic groups in the UK between 2001 and 2011 (Office for National Statistics, 2012).

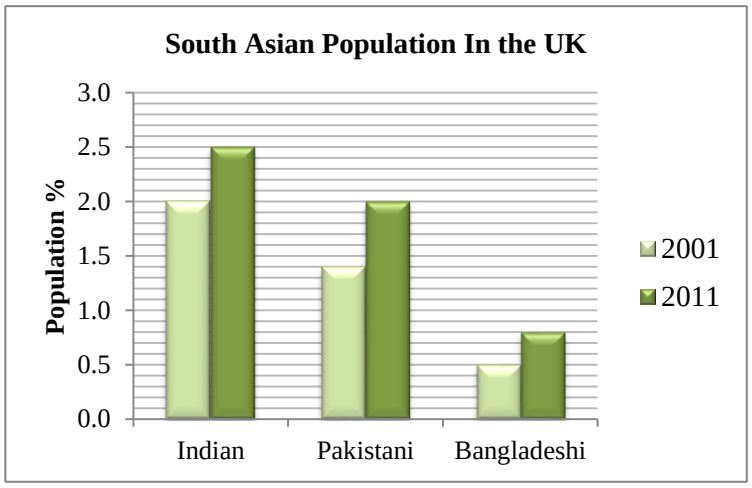


Figure 2-2 South Asian Ethnic Groups in the UK 2001 – 2011 (adapted from Office for National Statistics, 2012)

The Census data reveals that SAs live in all areas of the UK, clustering in certain areas reflecting migration patterns (Figure 2-3). For example, there is a high concentration of Indians and Bangladeshis residing in Greater London as well as the East and West Midlands; while, Pakistanis can be found predominantly in West Yorkshire, Manchester and the Midlands (Gill and Lloyd, 2009; Hanif and Karamat, 2009).

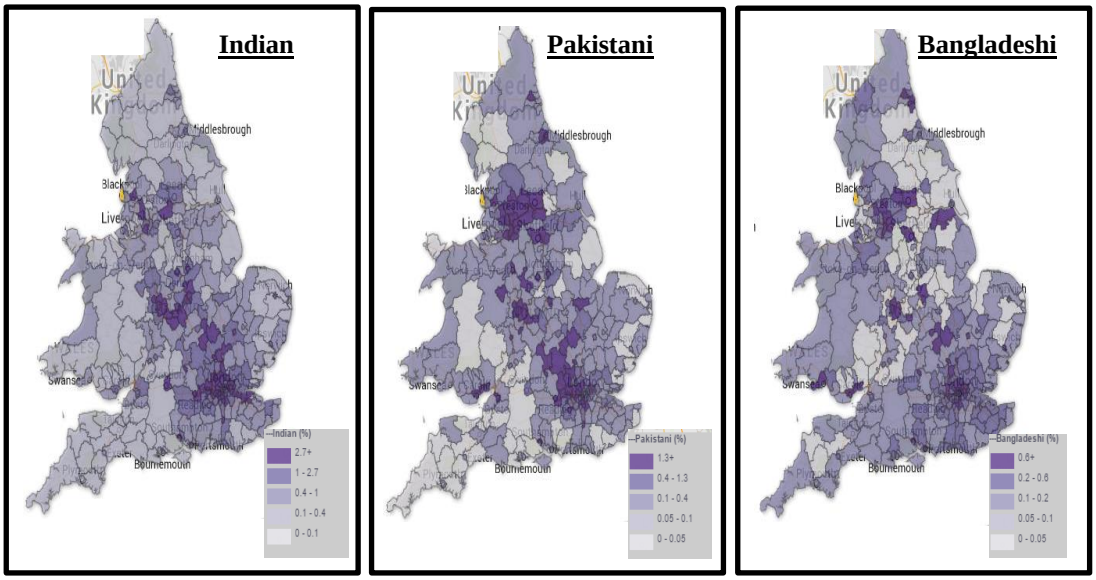


Figure 2-3 Distribution of Indian, Pakistani, and Bangladeshi Communities in the UK (adapted from Office for National Statistics, Census 2011)

2.4.2 South Asian Traditional Medicine

South Asian countries have a heritage of traditional herbal medicine; Ayurveda, Siddha and Unani are systems of traditional medicine (TM) native to SAs. Ayurveda is one of India's most well-known systems of TM which has hundreds of formulations; it was first documented in the *Rig Veda* around 1500 BC. Siddha is said to be the oldest system of medicine known, dating back over ten thousand years; it is another branch of traditional Indian medicine originating from South India. A newer concept was introduced around 1025 AD, by the Muslim herbalist Ibn Sina (more commonly known in the West as Avicenna), who took his inspiration from Greek and Islamic medicines to create Unani (Lone et al., 2012; Ansari and Satish, 2013).

2.4.2.1 Ayurveda

Ayurveda translates as '*the knowledge of life*' and is the holistic approach to traditional Indian herbal medicine; encompassing the use of HMs, breathing techniques, meditation, yoga and exercise as a complete system of managing health and preventing disease (Williamson, 2002). Ayurveda is believed to have originated from the Hindu God, Lord Brahma, known as the creator of the universe (Nerkar, 2007). It is thought to be one of the oldest systems of TM, which was developed more than five-thousand years ago; when the wise men known as '*rishis*', were thought to have retreated to the Himalayas to meditate to gain divine knowledge from Lord Brahma, about how to tackle diseases (Nerkar, 2007).

Their enlightenment was transcribed into *Vedas* which are Hindu scriptures, still referred to today. There are four *Vedas* in Hinduism; *Rigveda*, *Yajurveda*, *Samveda* and *Atharvaveda* which contain herbal remedies and cures for diseases.

The principles of Ayurveda originate from the first of the four scriptures known as the *Rigveda*. The *Rigveda* describes how the universe is made from five elements known as the *Pancha Mehabhutas*; space (*Aksha*), air (*Vayu*), fire (*Teja*), water (*Aap*) and earth (*Prithi*). In different combinations these elements make three forces: *vata*, *pitta* and *kapha*, which compose the human body and are known as the *tridosha* (Patwardhan et al., 2005). Williamson (2002) recognised that modern science has been able to interpret the three components of the *tridosha* as different systems within the body. *Vata* is equivalent to the central nervous system which regulates breathing, heart rate, and movement. *Pitta* represents the endocrine system and is involved in thermogenesis, digestion, and energy production. While the *kapha* corresponds to the immune system, providing protection to the body and wound healing. Disease occurs when components of the *tridosha* become imbalanced. For instance altered metabolism due to *pitta* imbalance links to thyroid gland malfunction (Godagama, 2001; Williamson, 2002; Patwardhan et al., 2005).

Ayurveda focuses on maintaining good health and making the body more resistant to illness and disease; equal importance is also given to the treatment of disease (Vishnuprasad et al., 2013). Ayurvedic practice takes into consideration individual differences and the treatment is tailored (Godagama, 2001). Ayurvedic medicinal plants have already provided the Western world with several active molecules for treating an array of conditions including: curcumins for inflammation, and guggulsterone for hyperlipidaemia (Patwardhan, 2000). Approximately six hundred plants are described in the Ayurvedic *Matria Medica* (Dash and Kashyap, 1980). Ayurvedic medicinal plants are attached to five key aspects; identification (ensuring the correct species is used is of paramount importance), cultivation, harvest, storage and usage; these factors can ultimately affect the activity, effect, and potency of the remedy (Godagama, 2001). The distinctive details outlining the selection and use of plants in Ayurveda ensures the correct plants are selected for the appropriate use; an innate system for controlling production of herbal remedies.

Within Ayurveda, plants are formulated into polyherbal mixtures known as '*rasayana'*. A common Ayurvedic mixture is *Chyawanprash*, believed to replenish the bodies' nutrients, containing a combination of over forty natural herbs including: *amla* (Indian gooseberry), cinnamon, clove, turmeric, asparagus and beetroot; according to Dabur Research Foundation (1991), the market leader of *Chyawanprash* sales, this blend of herbs which has been used for over two and a half thousand years, has antioxidant properties and helps strengthen the

immune system. *Triphala* is another common *rasayana*, consisting of three fruits: *amla* (*Phyllanthus emblica* L. Indian gooseberry), *beleric* (*Terminalia bellirica* (Gaertn.) Roxb bastard myrobalan), and *harad* (*Terminalia chebula* Retx. black myrobalan). This formulation has been used to treat numerous ailments including digestive problems, constipation, diabetes, hypercholesterolemia, hypertension, and cardiovascular disease (Ponnusankar et al., 2011).

In the sixteenth century during the colonization of India by the British, the traditional form of medicine was deemed backward and inferior and was gradually succeeded by Western medicine (Dash and Kashyap, 1980). Modern Western medicine was not accessible to all, it was expensive and difficult for those in rural areas to access; hence, in remote villages Ayurvedic traditions continued to thrive. Traditional doctors preserved the practices of prescribing and dispensing HMs. In 1980 the National Congress of India decided Ayurveda should be regulated just like Western medicine so institutes for training Ayurvedic practitioners were introduced (Godagama, 2001). In the late 1980's Ayurvedic remedies were being used in Western countries. In India, Ayurveda remains as the system for primary health care, and interest across the world is growing (Sharm et al., 2007; Lallanila, 2015). Yumi et al. (2008) suggest the reason why Ayurveda is becoming more popular in Western communities is due to the increased rates of immigration of Asians from India. This is supported by Sandhu and Heinrich (2005) who also found that immigrants brought their traditional forms of herbal medicine with them.

2.4.2.2 Siddha

Siddha is another branch of traditional Indian medicine which is believed to have originated more than ten thousand years ago. The Siddha scriptures are written in Tamil; hence, are predominantly used by people of Tamil origin (Krishnamurthy and Mouli, 1984). One of the distinctions made between Ayurveda and Siddha is that, Ayurveda is generally used more by people from the north of India and Kerala, while Siddha remains prevalent amongst those further South in Tamil Nadu (Zysk, 2008). Siddha healers still play an important role in rural health care (Pandikumar et al., 2011).

Legend has it that Siddha was created by the Hindu God, Lord Shiva, who passed the knowledge of medicine to the saints known as *Siddhars*. There were eighteen *Siddhars* - men who had achieved supernatural abilities, which enabled them to heal the sick, through their spirituality and meditation. These saints were believed to be able to cure any illness by utilising their divine knowledge to produce medicines from plants. Siddha takes into account

the individuals' disease; considering the persons symptoms, age, gender and environmental factors to produce a holistic treatment. The tongue, eyes, pulse, and urine are all assessed to determine the type of imbalance and to formulate the appropriate treatment (AYUSH, 2012).

There are several similarities between Ayurveda and Siddha. For example, the five elements which create the body: earth, water, fire, air and space which must be balanced. The concept of the three humours (Tamil: *tiritocam*, Sanskrit: *tridosha*) representing the wind (*vata*), bile (*pitta*) and phlegm (*kapha*) are also the same (Godagama, 2001; Zysk, 2008). The majority of the plants are used for similar conditions; some of the common examples include ginger for digestive complaints, tulsi for skin conditions, and garlic as an antibacterial (Chellappandian et al., 2012). The key differences recognised are that Siddha relies heavily on astrology to decide the appropriate treatment regime. The formulation process of medicines is deemed highly significant for example: the part of the plant used, the direction the sun penetrates the plant and the harvesting are all carefully regulated by the Siddha practitioners; deviations are believed to distort the effectiveness of treatment (Kumar et al., 2013). Ultimately both Ayurveda and Siddha provide a holistic system of healthcare; providing a path for life, promoting resilience to disease, and treatment for illness.

Siddha remains the primary health care regime for the majority of South Indians in Tamil Nadu; and is also practiced in Sri Lanka, Singapore and Malaysia (Chellappandian et al., 2012). Although modern medicine may be available across India, these systems of TM continue to thrive. Historical, cultural, and ecological factors could all contribute to the continued use of TM not only in India, but around the world (Kunwar et al., 2010; Pandikumar et al., 2011).

2.4.2.3 Unani

Unani is a newer form of TM, in contrast to Ayurveda and Siddha. Adopted by the Arabs, from the teachings of the ancient Greek and Roman philosopher-physicians Hippocrates and Galen. Having emerged over two thousand years ago (Lone et al., 2012), it incorporates traditional herbal remedies from several countries, including: India, China, Egypt, Persia, and many other Middle Eastern countries (AYUSH, 2012). The system was introduced to India by the Arabs, and used predominately by Muslims who lived there (Zysk, 2008). During the British rule, as with all the other forms of TM, Unani was deemed inferior and the availability of Western medicines surpassed the Unani system of healthcare.

According to Unani principles, disease occurs as a natural process. The system is based on Hippocrates' Humoral Theory; this suggests there are four humours in the body – phlegm

(*balgham*), blood (*dam*), yellow bile (*safra*) and black bile (*sauda*). Every individual has their own unique humoral constitution; therefore, treatment has to be formulated specifically once the correct diagnosis is made. Pulse (*nubz*) measurement is the most common method for diagnosis, as well as examination of urine and stools (AYUSH, 2012). Unani recognises that social, economic, political, and environmental factors can affect an individual's health; reinforcing the importance of health promotion through adequate education, lifestyle and behavioural modifications (Husain et al., 2010; Lone et al., 2012). Unani offers a variety of ways of treating disease; regimental therapy, one of the most popular methods of treatment, includes: massage, exercise, diuretics and emesis, to rid the body of impurities. Diet therapy involves controlling food intake, food should be clean and fresh to prevent disease onset. Pharmacotherapy entails the use of naturally occurring medicines, and surgery - physically correcting abnormalities (AYUSH, 2012; Lone et al., 2012).

One of the most common products used in Unani is black cumin (*Nigella sativa* L. English: small fennel, black seed, Sanskrit: *kala jeera,* Arabic: *Habbatul barak*). Seeds from the plant which is native to the Middle East and Southwest Asia are believed to be the remedy for all ailments (Tariq, 2008; Ansari and Satish, 2013). As defined by Islamic beliefs, it is said the Prophet Mohammed described the healing powers of black seed, as being able to cure any disease but death (Ansari and Satish, 2013). It is used for numerous health problems including: headache, cough, asthma, pain, rheumatism, diabetes, and many other conditions (Gali-Muhtasib et al., 2006; Tariq, 2008). The anti-inflammatory, anti-cancer, antibacterial, antifungal, and analgesic effects are being widely investigated by researchers (Al-Ghamdi, 2001). Thymoquinone is one of the active compounds known to have several health benefits, including analgesic and anti-inflammatory effects (Gali-Muhtasib et al., 2006; Hosseinzadeh et al., 2007). Pharmacological and toxicological investigations have shown that some of the active compounds extracted have hepatoprotective and nephroprotective effects (Ali and Blunden, 2003). The seeds can be eaten whole, the oil extracts can be applied to the skin or mixed with other ingredients to formulate a herbal concoction. The versatility, availability, and religious significance attached to this aromatic seed means it is often consumed daily, especially by the Muslim population (Ansari and Satish, 2013).

Like the other forms of traditional medicine discussed, Unani medicines have been used for centuries without real scientific validation. More research into the HMs recommended by Unani is required to investigate the safety and efficacy of this traditional medical system.

2.4.3 Evidence To Support the Use of Traditional Herbal Medicines

Traditional plant based remedies may be preferred to synthetic alternatives as they have been considered to be relatively safer, with better therapeutic effects, less side effects, more affordable, and easily obtainable (Ayyanar and Ignacimuthu, 2011). Hatfield (2007) states, "*Man's use of plants may be viewed as a long standing clinical trial; remedies that have stood the test of time are likely to be worth further investigation.*" Although there may not be much scientific evidence to prove the efficacy of HMs, some researchers claim the fact that they have been used for so long can be used to validate their use (Pandikumar et al., 2011). Despite the long standing use of HMs, a shift towards evidence based medicine, and scientific validation is prompting more research into HMs. Two commonly used examples which have now been researched extensively are turmeric and garlic. Turmeric (*Curcuma longa* L., Indian name: *haldi*) is commonly known as a spice, colorant and food preservative, which has several proposed medicinal benefits. It is said to have antioxidant, anticancer, antimicrobial and anti-inflammatory effects (Colalto, 2010). Curcumin, identified as the yellow pigment in turmeric, is a compound found in the rhizome of the plant; which is a therapeutically active component in turmeric responsible for its versatile medicinal properties (Sikora et al., 2010). Chemotherapeutic agents inhibit the hyperproliferation of cells, which develop into cancer. Synthetic drugs are often expensive, difficult to synthesise and have multiple side effects. On the other hand, curcumin extracted from turmeric is cheap, widely available, and is believed to kill tumour cells as it interferes with several cell signalling pathways that cause cell death by apoptosis and necrosis (Ravindran, et al., 2009; Sikora et al., 2010).

Garlic (*Allium sativum* L., Indian name: *lasan*) is another popular ingredient used in many herbal formulations; which is believed to have antibacterial, anti-platelet, and anti-hypertensive effects. The therapeutically active components recognised in garlic are allicin and alliin (Colalto, 2010; Garodia, 2006). A review by Tovar and Petzel (2009) analysed the efficacy of garlic for treating dyslipidaemia, and its action in reducing high cholesterol by inhibiting the enzyme involved in cholesterol synthesis; 3-hydroxyl-3-methylglutaryl-coenzyme-A-reductase. Garlic is effective at reducing low density lipids and triglycerides which contribute to high cholesterol; accordingly the benefits of reduced cholesterol correlate to reduced risk of stroke and heart disease. The antibacterial action of garlic was also observed in the West by the renowned scientist Louis Pasteur in 1858, who noted that bacteria died when they were exposed to garlic; crushed garlic juice was also used during the Second World War in wounds to prevent infections (Taylor, 2007; Arora and Kaur, 1999; Williamson, 2002). The mode of action is believed to be due to the active molecules, allicin

and alliin which interfere with thiol groups on bacterial and fungal enzymes, affecting essential metabolism leading to cell death, thus eradicating the organism (Williamson, 2002).

There are numerous HMs which are getting recognition for their pharmacological effects in the Western world. Dentists commonly recommend cloves (*Eugenia caryophylatta* Thunb., Indian name: *long*) to their patients for toothaches. The dried flower buds have powerful anaesthetic, analgesic, antiseptic, antimicrobial and anti-emetic effects (Chevallier, 2007). Eugenol (4-allyl-2-methoxyphenol) has been identified as the active substance responsible for many of the medicinal benefits of cloves. The culinary use of cloves is not only to add a spicy aromatic flavour, but traditionally it was added to prevent food spoilage and to aid digestion (Trajano et al., 2010). Another product which is being recognised for its cosmetic value is neem (*Azadirachta indica* A.Juss.); commercial soaps, shampoos, face washes, creams and lotions are now readily available. The neem tree is native to South Asia, and can grow up to thirty five meters tall; all parts of the tree can be used including the seeds, leaves and bark. It has been described as a pharmacy in its own right due to the many health benefits associated with it (Chevallier, 2007), including: antibacterial, antifungal, anti-inflammatory, anti-diabetic, antipruritic, anti-cancer and a blood purifying properties (Kumar et al., 2006; Boursier et al., 2011; Pandikumar et al., 2011).

The Foundation for Revitalisation of Local Health Traditions (FRLHT) in Bangalore, India, has established various projects across India to document the use of traditional HMs. One of the key priorities is to build a bridge between the traditional use and knowledge of HMs and modern scientific advances. By engaging in clinical research the FRLHT aims to establish clinical theories to support traditional use of HMs. By working with local communities, farmers, traditional healers, schools and educational institutes they have tried to preserve cultural heritage, cultivate sustainable resources, and provide an evidence base for traditional Ayurvedic practices (FRLHT, 2015).

2.4.4 Ethnopharmacological Research in the UK

Ethnopharmacology is "*the scientific study of ethnic groups and their use of drugs*" (Johnson and Sargent, 1996). Ethnopharmacological research involves a multi-disciplinary approach whereby botany, chemistry, pharmacognosy, pharmacology and other disciplines are considered to document traditional knowledge and use of medicinal plants (King, 1992; Patwardhan, 2005; Heinrich et al., 2006). The research methods used are diverse and tailored to the objectives of the investigation (Elisabetsky and Etkin, 2009). Plant material may be collected, identified and examined by botanists and other scientists while social science

inquires may be conducted to explore the medicinal ethnography (Elisabetsky and Etkin, 2009; Mukherjee et al., 2010). By combining methodologies from several disciplines such as social and natural science the objective of ethnopharmacological research which sets out to explore and understand traditional systems of medicine can be attained (Elisabetsky and Etkin, 2009).

There is a vast amount of ethnopharmacological research which has explored the use of traditional HMs by people around the world (Chhetri, 1994; Thring and Weitz, 2006; Malla and Chhetri, 2009; Kunwar et al., 2010; Ayyanar and Ignacimuthu, 2011; Bhatia et al., 2014). Pieroni et al. (2010) claims that most ethnobotanical research is conducted in exotic places and that a gradual shift towards exploring what is going on in '*back yards and urban environments'* will occur. They go on to say that ethnobotanists began to explore the use of plants by migrant populations in the late 1990s in North America; this was driven by an increasing interest in the use and perception of traditional HMs. In the UK, surveys to explore the use of HMs have been conducted (Ipsos MORI, 2008; Mintel, 2009); however, there is limited research into the use of HMs by SA communities in the UK (Bhopal, 1986a; Sandhu and Heinrich, 2005; Pieroni and Torry, 2007; Pieroni et al., 2010).

Bhopal (1986a) conducted interviews with 65 Asian participants in Glasgow to explore the role of TM in an Asian community. He discovered that the knowledge of HMs amongst participants was high, but HMs only had a modest role in participants' healthcare. Participants did not feel it was necessary to disclose information about their use of HMs to their HCPs; so Bhopal explored HCPs views of HMs too. He offered general practitioners (n=33) and healthcare workers (n=10) the opportunity to complete a questionnaire about Asian patients' use of HMs. The results revealed that HCPs awareness of Asian HMs was 'extremely low' and HCPs were unaware of their patients' use of HMs. As the research was conducted on a small Asian community in Glasgow the results are not representative of SAs in the UK; in addition, as the research was conducted in 1986 the results are now dated.

Sandhu and Heinrich (2005) explored the knowledge and use of HMs amongst a Sikh Punjabi community in London, by conducting semi-structured interviews with 84 participants. They documented the use of numerous HMs by the research participants and uncovered how participants managed minor ailments. As this research was focused on a specific religious (Sikh) and cultural group (Punjabi) the results are not representative of SAs; the authors do highlight that there is an urgent need to investigate the use of HMs amongst other ethnic groups in the UK.

The study by Pieroni and Torry (2007) focused specifically on Kashmiri, Gujarati, and English participants medical perception of five 'herbal drugs' (cinnamon, cloves, garlic,

ginger, and mint). A questionnaire was completed by 274 participants who were asked to state the perceived medicinal properties and uses of the products listed. They found a difference in traditional knowledge amongst the ethnic groups; whereby, Kashmiri participants had widespread knowledge of the herbal drugs listed while it was less prevalent amongst Gujarati and especially English participants.

Pieroni et al. (2010) conducted interviews (n=37) and focus groups (n=42) with Bengalis in Bradford to explore their use of traditional HMs. They recorded approximately 150 herbal preparations recalled by participants, and identified that many of them were still commonly used in the UK. Pieroni et al. (2010) found that middle-aged and older participants were more accurately able to recall HMs than younger participants; moreover, younger generations seemed to prefer CWM over traditional HMs. The results of this research indicate that HMs are still commonly used by Bangladeshi migrant communities in the UK.

The current research on SA communities' use of HMs in the UK is limited; but, it has provided a basis for conducting this research. Some of the concepts explored by other researchers will be further investigated in this study, to help gain a better understanding about the current use of HMs by SAs in the UK.

2.5 Methodology

2.5.1 Questionnaire Design

A questionnaire to meet the aims and objectives of the SATMED project was designed; to explore the current use of HMs amongst SA diasporic communities in the UK. Several draft copies of the questionnaire were piloted; Malhotra and Birks (2006) refer to pilot studies as, ‘*‘testing the questionnaire on a small sample of respondents for the purpose of improving the questionnaire by identifying and eliminating potential problems.’’* Amendments made to the questionnaire included: reducing the length of the survey without compromising the quality of the data, removing a question which used images of HMs which was ambiguous, and rewording some questions (Appendix 1). After which a more refined and tailored questionnaire was produced to meet the objectives of the research (Appendix 3). A participant information leaflet (PIL) was provided to participants to inform them about the purpose of the research (Appendix 4).

Initially the questionnaire was designed for participants to complete themselves; however, after conducting a pilot study the researcher believed it was more appropriate to administer the questionnaire personally. Several reasons for changing the way the questionnaire was conducted included: incomplete questionnaires, participants dropping out part-way, illegible handwriting, and feedback from participants who stated the survey was too long to write answers to despite having great knowledge of HMs. To ensure no bias was introduced from the researcher when asking the questions, thorough training from Professor Mark Johnson was attained; the training enabled the researcher to develop skills to keep participants engaged and affirmed how to ask the questions. Although this process was time consuming for the researcher the benefits outweighed the drawbacks. Advantages of the researcher administering the questionnaires included: the chance to build rapport with the participants – gaining trust and better responses to questions, a higher response rate, more complete questionnaires returned, and clarification of questions therefore less invalid responses (Dornyei and Taguchi 2010). Furthermore, as the researcher was fluent in Punjabi, Hindi and Urdu it was easier to engage participants who could not understand, read or write English.

A mixture of open and closed (quantitative and qualitative) questions were used in this survey. One of the reasons for using quantitative questions was so the data obtained could be statistically analysed; enabling the quantification of data and comparisons to be made. While the qualitative questions obtained richer, more in-depth knowledge of the individual participants’ perception regarding the objectives of the study (Levin et al., 2008). The questionnaire took on average ten to twenty minutes to complete.

2.5.2 The Target Research Population

The participant inclusion criteria for this research stated participants must be over eighteen years of age (so they could consent for their own participation without the need for parental consent), and from a South Asian (SA) background (i.e. Indian, Pakistani, or Bangladeshi) (Office for National Statistics, 2012). There is no existing database which permits randomised or systematic identification of a suitable population of SA origin (i.e. the sampling frame); therefore, an opportunity sampling technique was used. For this reason there was no pre-defined target of gender or age group to survey. Malhotra and Birks (2006) say opportunity sampling involves, *''being in the right place at the right time.''* The sampling method used followed established practice in other similar studies whereby the target research population was directly approached in anticipation of recruiting more participants (Sandhu and Heinrich, 2005; Thring and Weitz, 2006; Pieroni et al., 2010). Participants were reassured that all responses would remain anonymous (as no personal identifiable data was taken), and confidential. All the responses were kept in the possession of the primary researcher with no unauthorised access.

A unique aspect of this study was that it focused on SA populations as a whole; whereas, most other studies focused on single ethnic or religious groups (Sandhu and Heinrich, 2005; Jennings, 2014; Pieroni et al., 2010).

2.5.3 Ethical Approval

De Montfort University (DMU) ethical approval was obtained before the questionnaires were distributed (Appendix 1). The questionnaire and PIL (Appendix 3 & 4) were reviewed by the DMU ethics committee, who gave feedback and some minor suggestions. Instead of using the phrase 'side-effects' they recommended using a more neutral statement such as 'unwanted' or 'unexpected' effects. The ethics committee also commented on the length of the survey, which after the first pilot study was shortened; as some questions were removed or merged together.

2.5.4 Questionnaire Distribution

In total two hundred questionnaires were conducted across several locations in Birmingham and Leicester (one hundred in each city); two cities the primary researcher knew well (Table 2-1). The Census statistics suggested that Birmingham and Leicester would provide a reliable base for the field work as 7% of the population of England are now of SA origin; hence, the data would have wider applicability (Office for National Statistics, 2012).

Statistics revealed that over 30% of the residents in Birmingham are from an ethnic minority group. The SA communities of interest in Birmingham have increased from 17.9% in 2001 to 22.5% in 2011 (Office for National Statistics, 2012).The demographic profile of Leicester, in 2007, revealed that approximately 40% of Leicester's population were from an ethnic minority background (Leicester City Council, 2007). The 2011 Census suggests that the minority population of Leicester has risen to 54.9%, 35.8% of SA background (Office for National Statistics, 2012).

Table 2-1 Summary of Where Questionnaires Were Distributed

Date:	Location:	Number:
September 2013	Sachkand Nanak Dham International – multicultural temple in Birmingham	22
September 2013	Gheeta Bhavan Mandir Handsworth – Hindu temple in Birmingham	14
October 2013	Apna Ghar – Asian Community Centre and Day Care Centre in Birmingham	28
October 2013	Shakti – Asian Day Centre in Birmingham	14
November 2013	Apna Ghar – Asian Community Centre and Day Care Centre in Birmingham	13
February 2014	De Montfort University Campus – Leicester	23
May 2014	East West Community Project Day Centre – Leicester	11
June 2014	Santosh Day Care – Leicester	10
June 2014	Belgrave Neighbourhood Centre – Leicester	10
October 2014	De Montfort University Campus – Leicester	21
November 2014	De Montfort University Campus – Leicester	34

2.5.5 Questionnaire Analysis

The Statistical Package for the Social Sciences (SPSS) software, version 21, was used to input the quantitative data from the questionnaires and analyse the results. SPSS has multiple advantages over other analytical software including: the range of statistical techniques which enable exploration of relationships between variables (e.g. T-tests and analysis of variance test). In addition, significant differences between variables can be tested and cross-tabulations can also be created (Pallant, 2007). The software enables data to be coded; thus, allowing both quantitative and qualitative responses to be analysed. Microsoft Excel 2010 was used to create the graphical representations. The number (n) of participants or responses will be referenced throughout the results.

2.6 Results and Discussion

This section will summarise the results of the SATMED questionnaire. The survey was split into four segments: the first section explored participants' demographics, the second looked at the use of traditional herbal remedies, the third at the use of prescription medication, and finally the treatment of minor ailments. Although, two hundred surveys were conducted across several locations in Birmingham and Leicester, eight were discarded as they were either incomplete or the participants did not come under the SA inclusion criteria, leaving a total of one hundred and ninety two surveys for analysis.

2.6.1 Sample Description

This section was designed to determine participants' age, gender, ethnicity, religion, and occupation. In addition, participants were asked where they currently lived, were born, and where their parents were born to establish migration patterns and links between their knowledge of herbal medicines (HMs) and their heritage. A total of 192 questionnaires were used in this analysis.

2.6.1.1 Gender and Age Profile of Participants

The sample was comprised of 69% (n=132) female and 31% (n=60) male participants. As an opportunity sampling technique was employed, there was no method of screening or selecting the research sample to ensure an equal number of female and male participants were selected. One of the reasons for having more female respondents was due to the locations where the research was conducted; in the community and day centres visited in both Birmingham and Leicester it was observed that there was always a disproportionately larger number of females in comparison to males. Bailey (1994) suggested the gender of the researcher may affect participants' willingness to take part in research, saying SA male participants may not be as responsive to a female researcher. As there were fewer opportunities to approach male participants it is difficult to say whether or not the gender of the researcher affected the recruitment process.

There was a mixture of participants from the different age groups defined; as illustrated in Figure 2-4 which summarises the participants' age and gender profiles. A larger number of participants (n=59) were in the 61 years plus age range; this could be explained by the locations selected to target SA participants (i.e. day centres, community centres, and religious institutes) which were predominately used by older SA cohorts. In order to explore the younger generations' knowledge and use of traditional HMs places where younger SA

participants congregated were also identified; for example, the De Montfort University campus and library. There is much debate on how to classify age groups as young, middle aged, and elderly (WHO, 2010). For the purpose of this study those under 30 years will be referred to as young participants, middle age will include 31 to 60 year olds, and those over 61 years will be classed as older or elderly participants as identified by the Department of Health (2001).

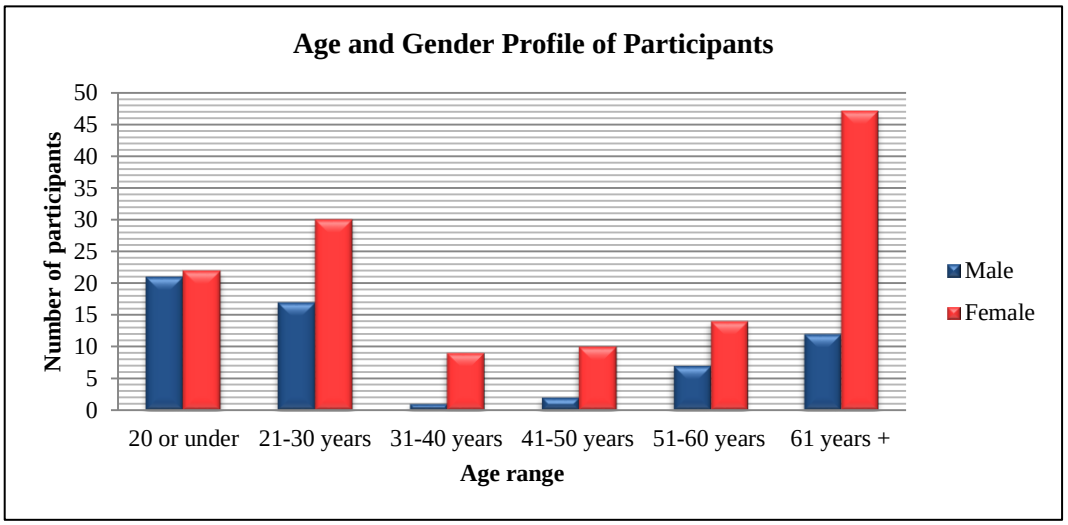


Figure 2-4 SATMED Participants' Age and Gender

2.6.1.2 Participants' Ethnicity

The 2011 Census results confirm the largest SA ethnic group in the UK are the Indians, followed by Pakistanis and a significantly smaller number of Bangladeshis (Office for National Statistics, 2012); this was also evident in this research sample (Figure 2-5). There were 73% Indian, 19% Pakistani, and 3% Bangladeshi participants. Questionnaires were distributed in areas where a variety of South Asians (SAs) could be found (e.g. SA community centres and the university campus). It should be noted that there was a large number of participants who would describe their ethnicity as Indian even though their families now reside in Pakistan and Bangladesh. In addition, several participants wanted to add the option of Asian British; however, it was explained to participants that their place of birth would be where they can express their nationality; but ethnicity was to determine their racial ancestry (Prabhat, 2010). Prabhat (2010) defines a person's ethnicity as being linked to their traditional and cultural heritage, language, rituals, behavioural and religious characteristics.

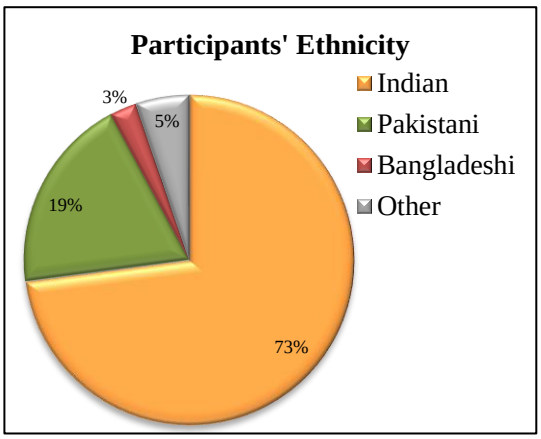


Figure 2-5 SATMED Participants' Ethnicity

2.6.1.3 Participants' Place of Birth

Figure 2-6 represents the birth place of participants and their parents; it highlights that 44% (n=84) of participants were born in the UK, 27% (n=52) in India, 17% (n=33) in Africa (Uganda, Kenya, and Tanzania), 10% (n=20) in Pakistan, and 2% (n=3) in other countries which included France, Germany, and Saudi Arabia. The majority of participants born in the UK were in the young age group (n=73); while those born in India (n=46), Africa (n=29), and Pakistan (n=15) were predominately in the middle age or older age group.

More than half of the participants' parents (55%, n=107) were born in India; despite this many of these participants families are now settled in Pakistan and Bangladesh but they still call themselves Indian as their parents were born in India before the partitioning into independent states (Healy and Aslam, 1989; Brown, 2006).

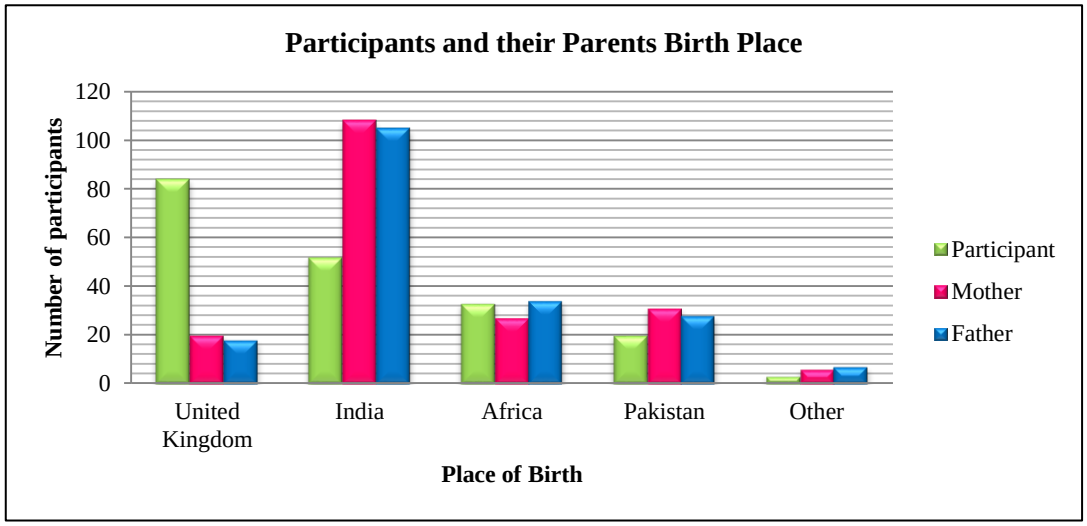


Figure 2-6 SATMED Participants' and Their Parents' Place of Birth

This information can be used to determine whether participants are first or second generation citizens in the UK. The term first generation could refer to citizens born in a country or those who were born in a foreign country and moved to take residence in another (Moffett, 2015). For the purpose of this analysis, first generation immigrants will be used to describe those participants born in a foreign country but now living in the UK. From the total sample population 108 participants can be identified as being first generation immigrants as they were born outside of the UK. Nine participants stated both parents were born in the UK, making these participants first generation citizens or natives to the country of birth. Second generation refers to people who are born in the country of relocation (i.e. the UK) with one or both parents born abroad (Moffett, 2015). There were 75 participants who were second generation citizens as they were born in the UK, but one or both parents were born elsewhere. Many of the participants' parents were born in South Asia and may be responsible for transmitting valuable knowledge of THM to future generations.

2.6.1.4 Participants' First Language Spoken

The primary language spoken by 50% (n=97) of participants was English (Figure 2-7). Followed by a quarter of participants who said their first language was Punjabi; this included people from India and Pakistan as prior to the partitioning of India, Punjabi was the main language spoken in the North. In the UK, Punjabi is the third most popular language spoken, followed by Urdu, Bengali, and then Gujarati (Office for National Statistics, 2012). In this study, Gujarati was the third most common primary language spoken and often these participants did not speak any other language. Other first languages spoken included Mirpuri and Tamil. Participants were also asked if they spoke other languages too. Many of those who spoke English as a first language also spoke Punjabi, Hindi, Urdu, and Gujarati. Other languages participants were fluent in included: Swahili, French, German, and Dutch.

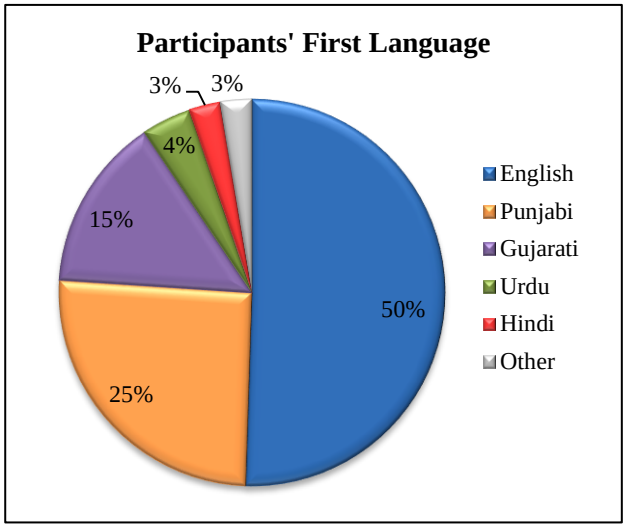


Figure 2-7 SATMED Participants' First Language

2.6.1.5 Participants' Religion

There were a fairly even number of participants from the three main SA religions: Hinduism (34%, n=64), Sikhism (26%, n=50), and Islam (36%, n=70) (Healy and Aslam, 1989). There was a small fraction of participants who said they were Christian and their parents were originally from South India; this is where Christianity is most common in India. The option of '*No Religion*' was only selected by 2% (n=4) of participants (Figure 2-8). Some religions have a strong affiliation with herbal medicines (HMs); for example the Tulsi plant represents a goddess in Hinduism (Miller and Miller, 2003). By determining participants' religion the link between religion and transfer of knowledge of HMs can be explored.

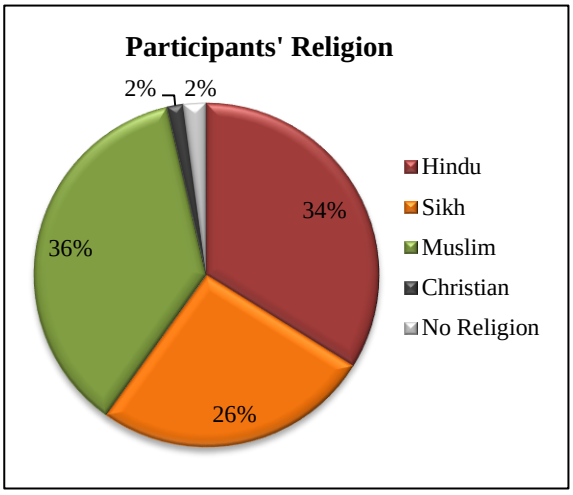


Figure 2-8 SATMED Participants' Religion

2.6.1.6 Participants' Qualifications

Figure 2-9 represents the highest levels of qualifications attained by participants organised according to the various age groups. The majority of participants who selected '*no qualification*' were first generation immigrants (n=50) and in the 61 years plus age range (n=42). Many participants who had some qualifications from India or Pakistan were considered to be equivalent to UK GCSEs; hence, a number of participants (n=10) in the 51-60 and 61+ age group had GCSEs. A-levels were the highest qualification attained for the majority of participants in the 20 or under and 21-30 years age range (n=69); 93% (n=64) of participants doing A-levels were currently enrolled on an undergraduate degree programme (e.g. pharmacy, nursing, psychology, business information technology, or accounting). Apprenticeships were more common amongst the middle aged population (n=14). Hanif and Karamat (2009) highlighted that many of the first SA immigrants had little or no qualifications; this is also evident from the results of this survey. On the other hand, some first generation immigrants with degrees may have come to the UK in search of professional job opportunities. Most of the degrees obtained by SA participants were professional degrees. Masters and PhD qualifications were also attained by a small proportion of participants.

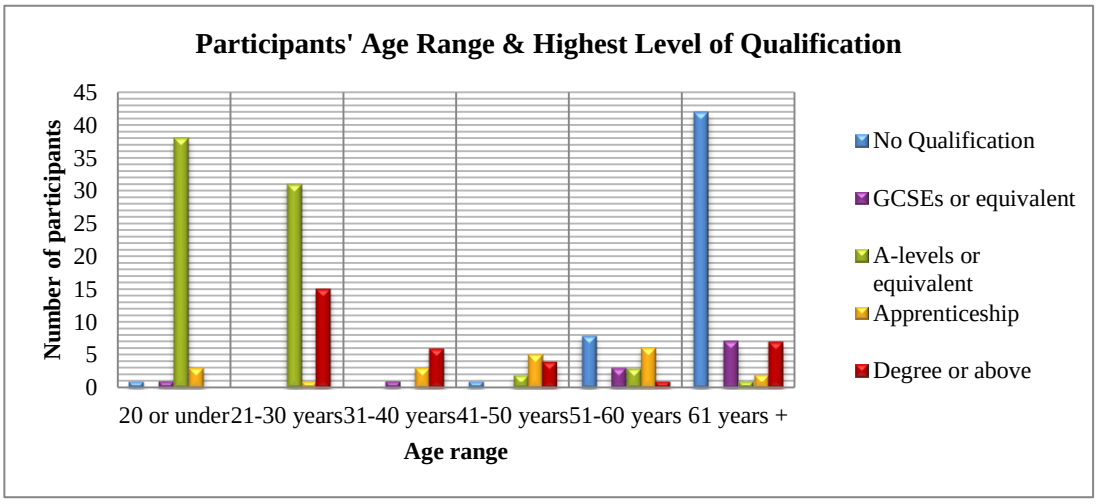


Figure 2-9 SATMED Participants' Age Range & Highest Level of Qualification

2.6.1.7 Participants' Occupational Status

Participants' occupations were summarised into the following categories: student, employed, unemployed, retired, or housewife. Students formed 38% (n=73) of the research population, followed by 27% (n=52) of participants who had retired. The employed population which comprised 24% (n=46) of participants worked in professional roles such as healthcare (doctors, pharmacists, nurses, and carers), finance (accounts) construction (surveyors), management (company directors and managers), and law (solicitors). Very few participants

worked in low or unskilled and trade jobs such as sales, or admin. 5% (n=10) of participants were considered to be unemployed for various reasons such as redundancy, disability, or cultural and language barriers which participants felt inhibited them from working. When middle aged SA women were asked about their occupation 11 participants (6%) said their job role was a "housewife". As this is not a paid occupation it was not included in the employed section instead it was given its own category.

2.6.1.8 Summary of the Research Participants

The research sample was composed of a diverse range of SA participants. There were more female than male participants from a variety of age groups, ethnic origins, religions, and cultural groups. Although the questionnaires were conducted around several locations in Birmingham and Leicester, participants living across the UK took part in this questionnaire (Figure 2-10); this was because the temples where the research was conducted had devotees from around the country coming to worship. In addition, the students at DMU were also from different parts of the UK. Participants from all over Birmingham, Coventry, Derby, Dudley, Leicester, Liverpool, London (i.e. Croydon, Hounslow, Ilford, Slough, Southall, Surrey, and Wembley), Loughborough, Milton Keynes, Northampton, Nottingham, Rotherham, Walsall, and Wolverhampton took part in this research (Figure 2-10). Due to the number of participants living across the UK it means the results can be generalised to SAs in the UK, rather than just SA communities in the two cities where the questionnaires were conducted.

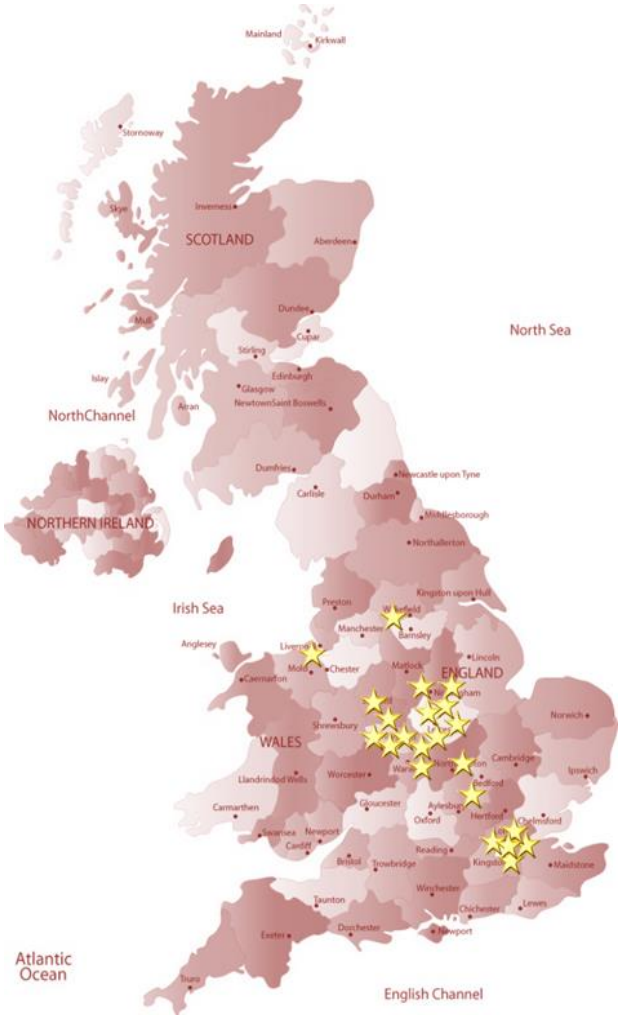


Figure 2-10 Map Highlighting Where Participants Lived in the UK

Adapted from http://www.map-of-uk.com/map.htm (Date accessed: 12/08/2015).

☆ Locations participants were from in the UK.

2.6.2 The Use of Traditional Herbal Remedies

Question 1: ***Do you use any herbs, plants, or spices to make homemade herbal remedies for your health? If YES, what do you use and why?***

The words herbs, plants, and spices were used to prompt participants to think about the different types of HMs they used. Two thirds of participants (66%, n=126) said they did use HMs to supplement their health and to treat various conditions. There was a vast range of HMs participants listed, including: *ajwain* (carom seeds) for digestion, ginger for nausea and coughs, aloe vera for eczema, cardamom as a mouth freshener or to aid digestion, mint for indigestion, fennel for coughs and constipation, and cloves for toothache. The numerous uses of turmeric recorded by participants included its use in treating coughs and colds, wound healing, as an antiseptic, anti-inflammatory, and its immune boosting properties. Honey and lemon was not only used for coughs and sore throats but also as a face mask to remove pimples and lighten the skin. Several products to control diabetes were identified such as curry leaf, fenugreek, karela, and tulsi. HMs to help reduce blood pressure (e.g. garlic) and cholesterol (e.g. cinnamon) were noted. Other conditions which participants claimed to treat with HMs included: healthy hair growth by using egg and yoghurt or henna, blocked sinuses opened with menthol or eucalyptus oil, and tea tree oil for spots and scars. Pieroni et al. (2010) highlight that very little is known about the use of homemade herbal medicines amongst migrant populations. The results of this question uncover that SA migrant communities in the UK commonly use a diverse range of HMs. The herbal products identified by participants have been summarised in Appendix 5. The 34% of participants (n=66) who said they did not use any herbal remedies were asked to move on to question 6 as the following questions were about participants' personal use of HMs.

For the following questions 2-5, the results are only for the 126 people who said they did use herbal remedies in question 1.

Question 2: ***Where do you get the ingredients for your herbal remedies from?***

This question was asked to explore how ingredients for HMs were sourced, and to determine whether or not ingredients for HMs could be bought in the UK. This question allowed for multiple responses as often people get their ingredients from a variety of different places.

Figure 2-11 illustrates the different ways participants obtained the ingredients for their HMs. From the analysis of the results, 21 participants said they got the

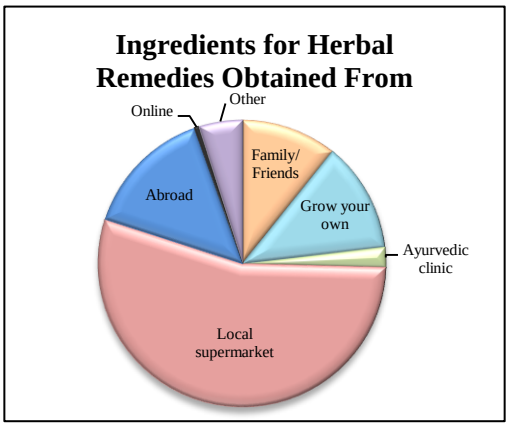


Figure 2-11 Where Ingredients for Herbal Remedies Were Sourced

ingredients from their '*family/friends*'. Participants mentioned family members grew some products so shared them, or parents and grandparents gave HMs if ever unwell as reasons for getting products from family and friends. Tulsi, *ajwain*, aloe vera, curry plant, mint, and coriander were identified as plants commonly grown by participants (n=23). A small number of participants (n=4) revealed they visited Ayurvedic practitioners and clinics for HMs. Three of the participants who selected the '*other*' option, disclosed that they got their HMs from '*Baba Ramdev*' they did not class this as an Ayurvedic clinic/ practitioner as there was a strong religious affiliation. Ramdev studied Sanskrit and yoga before he began teaching others; he became famous when his yoga sessions were aired on Indian television channels across the world. He established the Divya Yog Mandir which was supported by a pharmaceutical company (Divya Pharmacy), which formulated and sold Ramdev's HMs, all based on traditional Ayurvedic remedies (Divya Yog Mandir, 2015). These HMs are manufactured in India and sold around the world; evidently they are available in the UK with Ramdev centres open across the UK and several participants claimed to be using them. The remaining six participants who selected the '*other*' option said they got their HMS from: the pharmacy (n=2), Holland and Barrett, The Body Shop, and Forever Aloe Vera representatives.

Participants (n=27) also stated they got their ingredients from 'abroad' this included countries such as Africa, India, and Pakistan. Raw ingredients including *ajwain*, *shilajeet*, neem, tulsi, and turmeric; as well as commercial products such as *chyawanprash*, *churan*, and *amrat dhara* were commonly imported from India and Pakistan. Participant 67 said, *''when my family comes from Pakistan or Saudi Arabia they bring us some neem leaves,''* while participant 108 stated, *''I buy my herbs fresh in India, let them dry and grind them, then bring them back to the UK.''* Despite the widespread availability of turmeric in the UK, several respondents mentioned that their families back in India and Pakistan grew their own turmeric; which they believed was better quality and more effective as highlighted by participant 162, *''you can tell in the colour and taste of the turmeric it is much better from Pakistan.''* Pieroni et al. (2010) also found participants in their study imported ingredients for HMs from abroad if they were not available in the UK; but, mentioned that many ingredients were now available in Bangladeshi shops or larger supermarkets.

The most common place to get ingredients for HMs, selected by 101 participants, was local supermarkets. To explore the availability of ingredients in the UK participants were asked to specify whether they got their products from Asian supermarkets, Western chain supermarkets (e.g. Asda, Tesco or other Western shops) or both. Figure 2-12 exemplifies that a similar number of participants said they got their ingredients from either Asian shops only (n=35) or both Asian and chain supermarkets (n=36).

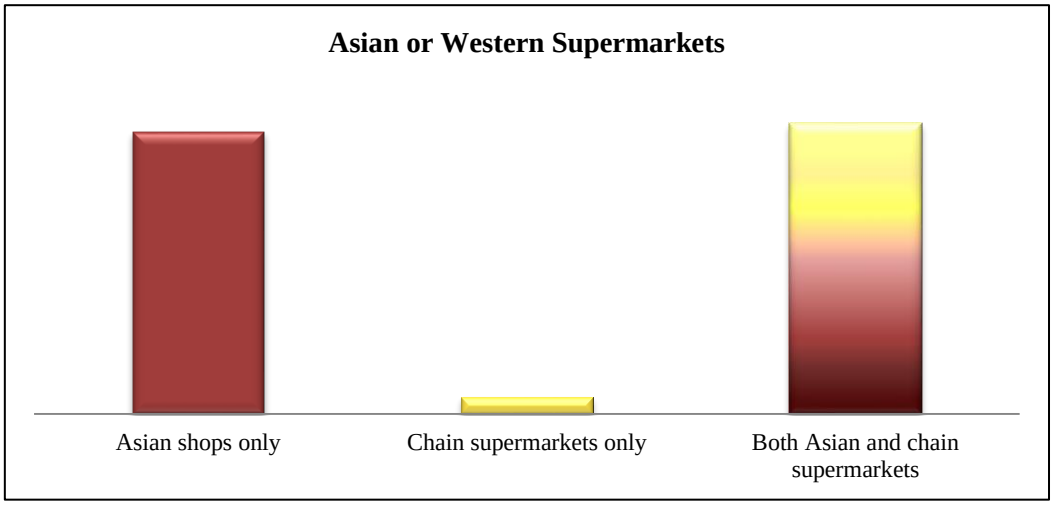


Figure 2-12 Where Ingredients for Herbal Remedies Were Bought From

Just over half of respondents (51%, n=18) who specified '*Asian shops only*' were over 61 years old. Specific Asian shops where the ingredients were bought from were mentioned for example in South Birmingham '*Metha'* and in Leicester the '*Kelani'* store. There could be a variety of reasons why older participants preferred only to visit Asian shops; for instance, they know the stores which stock the ingredients they want, and are able to communicate with people better in their own language. Language barriers could be a key factor in explaining why elderly SAs prefer to shop in Asian supermarkets. The Census 2011 highlights that the majority of the 2.4% of the population of Birmingham who cannot speak English are from a SA background (Birmingham City Council, 2013). Other participants from the various age groups who claimed to only get their ingredients from Asian supermarkets said they were familiar with the shops and trusted the quality of the products, and commented on the price of the products; participant 131 said, *''I prefer the Asian shops because they are cheaper.''* Participants made references to the past when products such as *gor* (jaggery), black seed, and *churan* were ordered from abroad when family and friends went to visit, as they were only available in their native countries; but, now the products were readily available in the Asian shops in the UK.

The two respondents who stated '*supermarket only*' said this as they claimed they did not have Asian shops nearby, so relied on whatever was available in their local area. Finally, those who stated '*both Asian and chain supermarkets*' commented on how ethnic ingredients were now available in Western chain supermarkets too; so went wherever was convenient. The large supermarkets are now catering for the needs of migrant communities by stocking ethnic ingredients (Retail Think Tank, 2013). As identified by the participants in this research, ingredients which were once only available from SA countries are now becoming more easily accessible from their local supermarkets; this could suggest a change in the way ingredients for traditional herbal remedies are sourced.

During the research period a satellite project to investigate the availability of traditional SA HMs in Leicester was explored in collaboration with Professor Adrian Slater, Dr. Caroline Howard, Dr. Tamsyn Thring and Dr. Ketan Ruparelia. A variety of herbal products were collected from several supermarkets and SA shops around Leicester and Birmingham. The sample inventory comprised a range of products including raw ingredients (e.g. fresh karela, ginger and turmeric), seeds, powdered and dried products, as well as preformulated herbal remedies (e.g. turmeric capsules, tulsi tablets, and *Chyawanprash*). While collecting samples it became apparent that the products mentioned by the participants in this research were readily available.

Based on work by Seethapathy et al. (2015) and Kumar et al. (2015) the research team explored the applications of DNA authentication on the herbal remedies collected. The study by Seethapathy et al. (2015) looked at authentication of medicinal plant laxatives *Cassia* L., *Senna* Mill., and *Chamaecrista* Moench. After collecting market samples of dried leaf, fruit, and bark material from shops across several locations in South India, they discovered the adulteration of species amongst the samples; leading them to raise concerns about the species of medicinal plants available in the Indian herbal market. Similar work by Kumar et al. (2015) also suggests there is a problem of species adulteration and substitution amongst the Indian herbal market. DNA authentication work on the herbal remedies collected in Birmingham and Leicester correctly revealed the species of the fresh samples collected. While other formulations collected such as tablets, capsules, dried turmeric root and neem leaves were not so easy to authenticate. Work to complete this study is still in progress.

One of the observations highlighted was the lack of information about the ingredients of products on the labels of some of the commercial formulations brought. Some products had very little information about the ingredients while others had none at all. Furthermore, several products which had passed their sell by date and others which had their expiry dates changed by hand were bought. This raises concerns about the quality of herbal remedies available in the UK and suggests further research in required.

Question 3: ***What form of herbal remedies do you use?***

Participants were given several options to select the types of herbal formulations they used (Figure 2-13). The most common types of herbal formulations used included: fresh herbs (e.g. mint, coriander, and fenugreek), dry powders (e.g. turmeric, cinnamon, black pepper, black salt, *hing*, and *churan*), and seeds (e.g. fennel, rye, mustard, black seed, and *ajwain*). Participants mentioned that as some ingredients were not readily available in the UK, they would import dried material from countries such as India and Pakistan; tulsi, turmeric and neem leaves were commonly mentioned as being imported to the UK. Liquid formulations used included: oils (e.g. olive, almond, coconut, sunflower, and mustard oil), syrups for coughs, and juices for diabetes (e.g. karela, and carrot juice). Tablets and capsules such as ginseng, Echinacea, senna, green tea, acai berry, and primrose oil were often bought from places such as Holland and Barrett, pharmacies, and other health shops. Ayurvedic and Chinese traditional herbal tablets for digestion (e.g. *Triphala* and *churan*), diabetes and weight loss were bought from India, Ramdev centres (both in the UK and abroad), and Chinese herbal shops. One of the participants who selected the '*Other'* option described a sap which is harvested from the mountains in India known as *Shilajeet* used to improve blood circulation, good for people who have had a stroke, and has many other health benefits associated (Wilson et al., 2011).

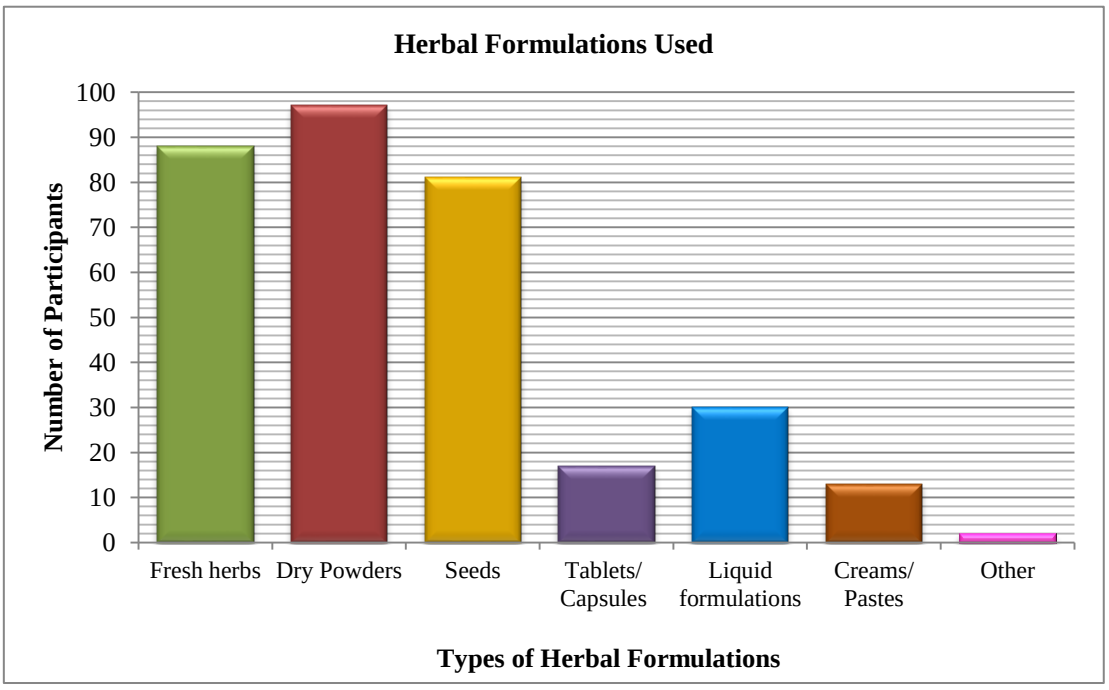


Figure 2-13 The Types of Herbal Formulations Used By Participants

Question 4: ***After using herbal remedies do you notice any unwanted effects? If YES please state.***

Of the 126 participants who stated they did use herbal remedies, 87% (n=110) said they did not experience any unwanted effects after taking HMs; while 13% (n=16) claimed they had. Generally, participants commented on how HMs were natural and so safer to use, with no side-effects. Ideologies that HMs were designed by God to help people heal themselves naturally were mentioned by participants in this survey. The notion that people think HMs are natural and therefore safe has been documented by many other researchers too (Newall et al., 1996; Ipsos MORI, 2008). The Medicines and Healthcare Regulatory Agency (MHRA) commissioned an investigation into the public's perceptions of herbal medicines in 2008; the report revealed that just one in ten participants were aware that some HMs have side effects (Ipsos MORI, 2008).

The unwanted effects reported by participants are summarised in Table 2-2. Some of the effects noted can be classed as minor as they are intermittent effects; for example excessive gas or wind produced by some herbals (participant 61) or a sore throat caused by the consumption of ginger (participant 72). Participant 42 claimed that adverse effects could be easily managed with the correct knowledge and gave the example of how the effects from '*garam'* things can be counteracted by taking some orange juice, which has an opposite effect. On the other hand, some adverse effects mentioned were more serious and potentially life threatening, as reported by participant 116, who reported fresh blood in his stools after consuming HMs.

Defining the concept of *garam* and *bhye*:

The term '*garam'* when translated into English literally means hot. *Garam* is a concept used by SAs to classify ingredients which warm up the body. In Ayurvedic and other traditional medical systems the term describes substances which heat the blood or warm the body temperature. Participants identified ingredients such as: *ajwain,* cumin, cloves, cinnamon, coconut, cardamoms, dates, garlic, ginger, karela, peanuts, and turmeric as being *garam*. According to the research participants, when *garam* ingredients are consumed in excess the side-effects of an overheated internal system can include: nose bleeds, dizziness, palpitations, sweating, itching, and gastric disturbances (e.g. diarrhoea or acid reflux). Nevertheless, the importance of consuming *garam* foods in proportion was highlighted for keeping the body healthy, and was useful for treating conditions such as arthritis and diabetes. Some *garam* foods should be avoided during pregnancy due to the risk of a miscarriage; for example, mangos and papaya during the first few months, while tulsi should be avoided throughout the entire pregnancy (Winston and Maimes, 2007). During the

distribution of the questionnaire several participants made reference to Western medicines as being *garam*. This may be because the side-effects from CWM are similar to those experienced after consuming *garam* substances, or because some of the active ingredients fall into the same category as other *garam* substances. Participant 2 claimed, ‘‘*paracetamol is garam and I cannot take too much, as if I do I start itching, especially in my head*!’’ Itching is a documented side-effect caused by paracetamol (Joint Formulary Committee, 2014).

There is no straight forward translation for the term *‘bhye’*. In contrast to *garam* ingredients which cause heat, *bhye* ingredients have the opposite effects; so Bhopal (1986b) refers to them as ‘cold’ substances. It is a term used to describe an internal imbalance, to which there is no equivalent term in Western medicine. Symptoms of *bhye* can include: nausea, feeling bloated, excessive gas production, increased salivation, and mucus production. Substances participants identified as being *bhye* include: rice, pulses, potatoes, carrots, turnips, melon, lemon, oranges, okra, and cauliflower. Once again it was highlighted by the research participants that these ingredients are vital for a well-balanced internal system but deficiency or excess can cause adverse effects.

Table 2-2 Unwanted Effects Experienced After Using Herbal Remedies

Participant Number:	Unwanted Effects From Herbal Remedies Reported by Participants:
20	*‘‘Too much of certain ingredients can cause side effects for example when I have pure honey and turmeric mixed together for a sore throat it gives a painful burning sensation.’’*
22	*‘‘Dushanda used for high temperatures gives me phlegm- because it is bhye.’’*
23	*‘‘Some types of masalas give me diarrhoea, as they are garam.’’*
29	*‘‘Mustard oil causes sickness/ allergy.’’*
40	*‘‘Too much neem can cause dizziness (feel faint and dizzy) so to counteract this side-effect have some ghee with it.’’*
42	*‘‘If I have too much garam food like ginger or garlic, I drink some orange juice because it is bhye so will balance the imbalance.’’*
47	*‘‘Taking too much ajwain makes me dizzy.’’*
61	*‘‘Some ingredients cause wind.’’* *‘‘Discolouring of teeth, e.g. haldi causes teeth to look yellow.’’*
65	*‘‘Karela juice is great for diabetes but it should not be too taken too often as it is strong and it can affect the liver.’’*
66	*‘‘Too much honey is bad for blood pressure.’’*
70	*‘‘I was getting hot flushes when taking saunf, ajwain and ginger mixture; because they are garam they warm the blood so they can cause hot flushes.’’*
72	*‘‘Too much adrak (ginger) causes irritation to the throat (throat feels like it is ulcerated)’’*
73	*‘‘Haldi caused stomach ache as it is garam in excess caused side-effects so had to stop it for a while.’’*

Table 2-2 Unwanted Effects Experienced After Using Herbal Remedies Continued…

82	*''Too much aloe vera can cause diarrhoea'*
105	*''Too much ginger powder (ganthora) which was used for cramps affected my stomach ulcer, as it is so garam.''*
113	*''Garlic gives me burps.''*
116	*''I got very bad diarrhoea and fresh blood in my stools after taking some Baba Ramdev medicines while in India. I felt like I was going to die and it was very scary. I called the Ramdev hospital where I was being treated for my diabetes and the herbal doctor just said stop taking the medicine immediately, which I did. After that experience I would never trust that medicine again, I do not know what could have happened.''* *''Feels very hot (body temperature) when has cinnamon, as it is garam.''*

From the analysis of the survey it should be noted that the definition of unwanted effects or side-effects may be different amongst SA and Western communities. During the distribution of the questionnaire it was obvious that some effects caused by the consumption of HMs were not accepted as side-effects. For example, if a remedy consumed for a headache caused GI disturbances (e.g. excessive belching, diarrhoea, or flatulence) it was believed the body was excreting the excess waste products from the body to resolve the ailment. Sometimes the undesirable effects were thought to be part of the process of recovery (Pole, 2008). In addition, there are differences in diagnosis, treatment, and side-effect profiles between cultures. This can be exemplified in the diagnosis and acceptance of various health conditions, such as mental health problems. Traditionally in SA communities the cause of mental health problems may have been due to black magic/ witchcraft/ evil spirits, or the will of God due to previous sins and bad deeds (Time to Change, 2010). The theories behind the cause of mental health and method of treatment are different in SA and Western cultures. This highlights that traditional and Western systems may perceive and treat illnesses in diverse ways. Due diligence is required when conducting research in SA populations as concepts can easily be misinterpreted. Whilst conducting the surveys the researcher was always mindful of such differences and asked the participants to explain concepts fully.

Question 5: ***Where did you learn about herbal remedies?***

Participants who said they used herbal remedies for their health, were then asked where they had gained this knowledge from (i.e. family, friends, GP, Pharmacist, educational institute, or other); 80% (n=101) of participants stated, 'from their family' (Figure 2-14). Knowledge of HMs was gained from both male and female family members. Participant 29 mentioned her grandfather was an Ayurvedic practitioner and participant 87 said her father was an Ayurvedic practitioner. However, most participants made reference to their mums as being the main source of learning about HMs. The transmission of knowledge of HMs has been passed on by word of mouth throughout history, from one generation to the next (Chhetri, 1994; Thring and Weitz, 2006; Bhatia et al., 2014). This reinforces the key role that family play in passing on this valuable knowledge.

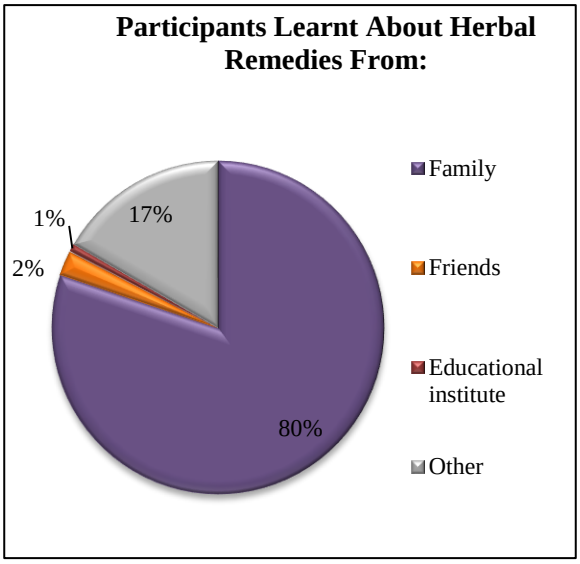


Figure 2-14 Where Participants Learnt About Herbal Remedies

Throughout the SATMED questionnaire any references to family and friends is combined in the option '*family/ friends*'; this is because in SA cultures friends are often referred to as family, as are neighbours and people from the same village. The distinction between family and friends is not always the same as in Western cultures. However, for the purpose of this question, to establish exactly where participants learnt about HMs, the option was split into individual components i.e. family or friends instead of family and friends. Just three participants (2%) specified that they had learnt about HMs from their friends. One participant (1%) said she learnt about HMs from her school in India, as it was part of the curriculum. The option of GP or Pharmacist was not selected by any of the respondents.

Participants who selected '*other*' as their option (17%, n=21) for this question, said they learnt about HMs from a variety of places such as: the television (in particular the Asian channels such as Astha which airs Baba Ramdev remedies, and ZeeTV), radio, internet (e.g. Facebook, Twitter, and YouTube), herbal shops (e.g. Holland and Barrett), traditional healers, and from their religion (two participants spoke about the value of black seed in Islam). There was some discussion amongst participants about how the media was introducing them to HMs. Several participants claimed while using social media websites

and applications, such as Facebook and Twitter, the adverts which pop up are often about HMs. Usually HMs for hair and weight loss were advertised; however, this had led the participants to look into other HMs of interest. Participants 120 and 123 explained that they often researched on the internet for natural remedies. As did participant 165 who said she often watched YouTube videos to see how to make various herbal facemasks for her acne. Kanthe (2010) suggests the media can have a significant role in influencing peoples' decision to try herbal products; this was true for some of the participants in this study.

The results from this question have revealed that the principle route of transmission of knowledge of HMs is from family. The traditional knowledge of HMs has often been passed on from parents and grandparents. Each family may have their own formulation, dosage, and duration of treatment which has been optimised through generations of traditional use (Bhamra, 2011). However, with advances in technology, popularity of social media, and the ease of access to a vast array of information, the way people are learning about HMs is changing (Hegg, 2013).

The following questions are inclusive of all 192 participants responses.

Question 6: ***Is herbal medicine part of your traditional family & cultural background?***

SA countries are rich sources of natural remedies and are renowned for their long standing use of HMs (Bhatia et al., 2014). Pieroni et al. (2010) claims that HMs are embedded in the cultural heritage of SAs and is a representation of identity of migrant communities. This question was asked to determine the current status of such traditions amongst SA populations in the UK, and to determine whether or not the knowledge of HMs is being passed onto generations born in the UK. WHO (2008) states that in some Asian and African countries more than 80% of the population still depends on traditional HMs for their primary health care. In this study the majority of participants are first generation immigrants (n=108) or second generation citizens (n=75) in the UK; therefore, it is assumed that HMs will be part of their traditional family background. The significant majority of participants in this research (85%, n=163) said HMs were part of their heritage; this suggests that SA communities in the UK are aware of the traditional use of HMs, regardless of whether or not they use HMs themselves (Figure 2-15).

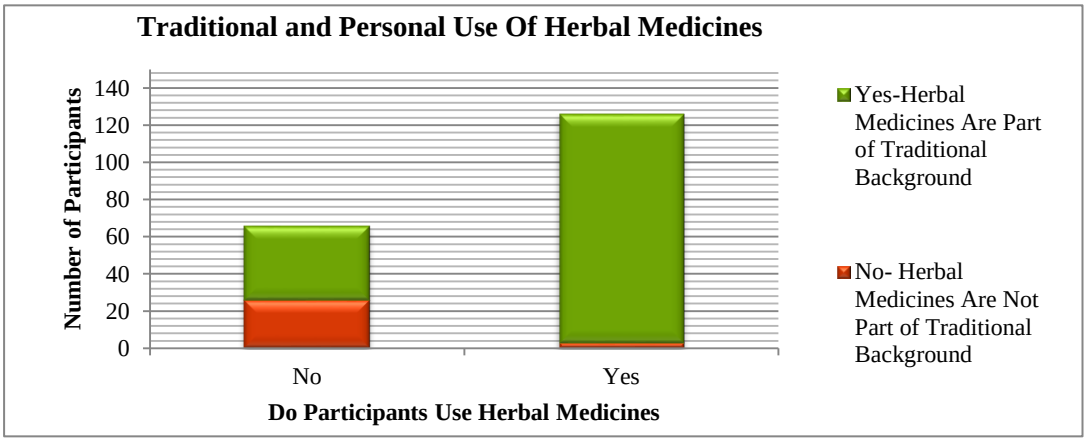


Figure 2-15 Participants Personal Use of Herbal Medicines and Traditional Heritage

Only 15% (n=29) of participants said HMs were not part of their traditional family background. Analysis of the results revealed that most of the participants who stated HMs were not part of their traditional family and cultural background were in the young age group (n=15, of which 12 participants were second generation citizens), born in the UK (n=16), with at least one or both parents also born in the UK. As only a small number of participants claimed HMs were not part of their traditional family background, claims such as a loss of transmission of knowledge to SAs in the UK cannot be made; although, during data collection one participants did suggest that this was the case. Participant 39 commented on how Westernised her own children had become that they did not even let her use HMs which she knew worked, they only used CWM and never considered the use of HMs despite it being part of their heritage. It was also noted that two participants claimed their families only ever used CWM; thus, they were not aware of traditional or cultural practices of HMs.

Question 7: *How often do you use herbal medicines?*

This question adopts a modification of the Likert scale to determine the frequency of use of HMs. Initially, the questionnaire was designed with the options: very frequently, frequently, occasionally, rarely and never; however, after review these options were changed to daily, weekly, monthly, when required, and never, to better determine the use of HMs.

Participants were asked how often they used HMs to determine how frequently they were used by the research sample (Figure 2-16). The majority of participants (48%, n=92) claimed to use herbal remedies '*when required*'. Remedies such as: cardamom pods for nausea, cloves for toothache, honey and lemon for coughs, and aloe vera for skin conditions were commonly used when required. The 26% of participants (n=50) who stated they used herbal remedies '*daily*' mentioned taking substances such as cinnamon to lower cholesterol, *ajwain* for digestion, *masala* tea and turmeric milk for general health and wellbeing. Out of these 50 participant who used herbal remedies daily just over half (56%, n=28) were elderly participants. The few participants (3%, n=5) who claimed to use herbal remedies '*weekly*' made reference to not taking too much of various ingredients as they are *garam* so should only be consumed in moderation; karela for diabetes and some *masalas* were amongst those that should be consumed in moderation.

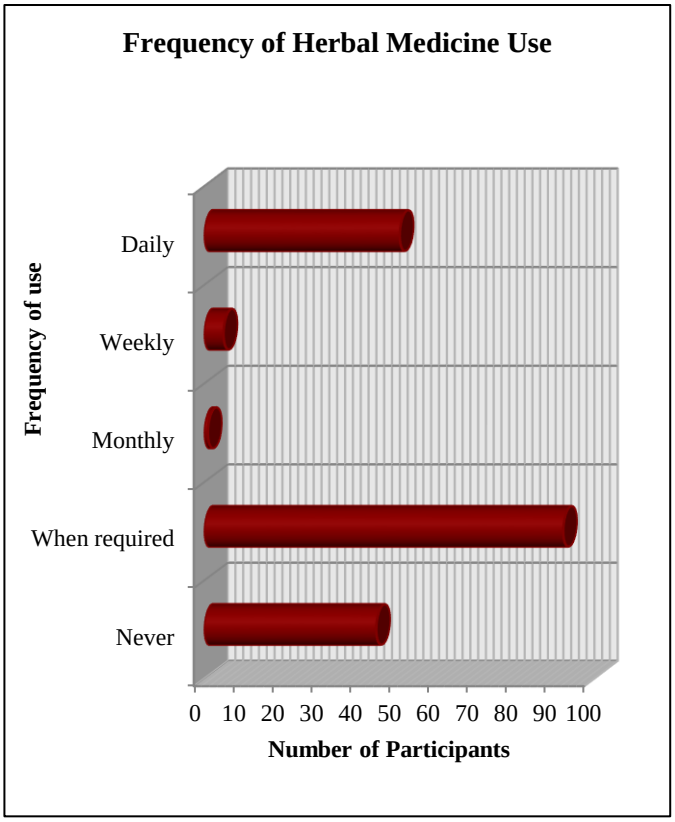


Figure 2-16 How Frequently Participants Used Herbal Medicines

Despite 66 participants claiming they did not use any HMs for themselves in the first question, only 44 participants said they '*never*' used herbal remedies in this question. This implies the 22 participants who selected alternative options may have later realised that they did sometimes use HMs. This would suggest that more of the research participants do actually use HMs than first thought.

The following question was divided into two parts; the first section (a) asks participants to list any herbal remedies, products, or ingredients they know which have medicinal benefits; while the second section (b) focuses specifically on medicinal plants.

Question 8A: ***List as many herbal remedies, products or ingredients you know of…***

The aim of this question was to explore participants' knowledge of HMs. The first draft of the questionnaire asked participants to list '*herbal remedies*' they knew of; however, after reviewing the feedback from the pilot study the terms '*herbal remedies, products or ingredients*' were used to stimulate participants to think about different HMs they knew of. The refined wording in the question acted as a prompt which permitted for a wider range of responses, without influencing participants' answers (Harris and Brown, 2010).

Participants were asked to list as many products as they could think of which were good for their health or had medicinal values, even if they did not personally use them. The results exemplify a vast range of herbal remedies known and used by the research sample (Appendix 5). Figure 2-17 represents the number of items recalled by participants; this includes the individual ingredients which may be used to formulate a herbal remedy and commercial products. Only 7% (n=13) of the research population were unable to list any herbal products in this question. The mean (M) number of responses per participant was seven. The highest number of products recalled was by participant 16 who listed thirty products.

The most popular product mentioned by 57% (n=109) of participants was turmeric. Other frequently mentioned items included: ginger, *ajwain*, fennel, cinnamon, garlic, aloe vera, clove, tulsi, black pepper, neem, and black seed. Various nuts (e.g. almonds and walnuts), teas (e.g. green tea, nettle tea, and masala tea), and oils (e.g. olive, mustard, eucalyptus, sesame, castor, sunflower, and fish oil), were also mentioned (Appendix 5).

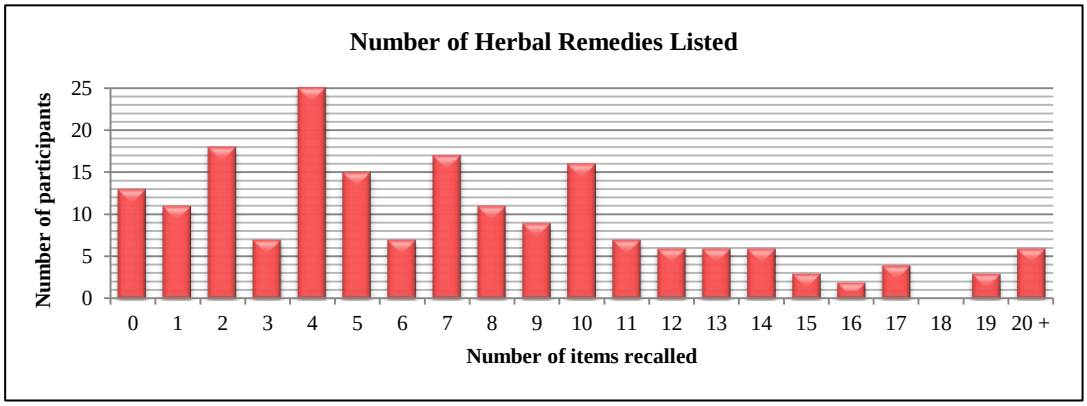


Figure 2-17 The Number of Herbal Remedies Recalled by Participants

Question 8B: ***What plants do you know that have medicinal benefits?***

Part B of the question asked participants to specifically name any plants which had medicinal benefits; Figure 2-18 represents the number of plants recalled by participants. The majority of participants (24%, n=46) recalled at least one medicinal plant (M = 2), the maximum number of plants listed by one participant was twelve. Aloe vera was the most frequently mentioned plant by 41% (n=80) of participants, followed by mint (28%, n=54) and tulsi (23%, n=44). Participants said they used aloe vera for skin conditions (e.g. eczema, itchy skin, burns, and skin irritation), diabetes, and arthritis. The gel contained in the fleshy leaves of the aloe vera plant has been associated with anti-inflammatory and wound healing effects which have been linked to the mannose-6-phosphate sugar component (Davis et al., 1994). Other traditional uses of aloe vera include its use in treating tooth aches, aiding the recovery of mouth ulcers, stomach and duodenal ulcers, irritable bowel syndrome, and its immune boosting effects (Chevallier, 2007)

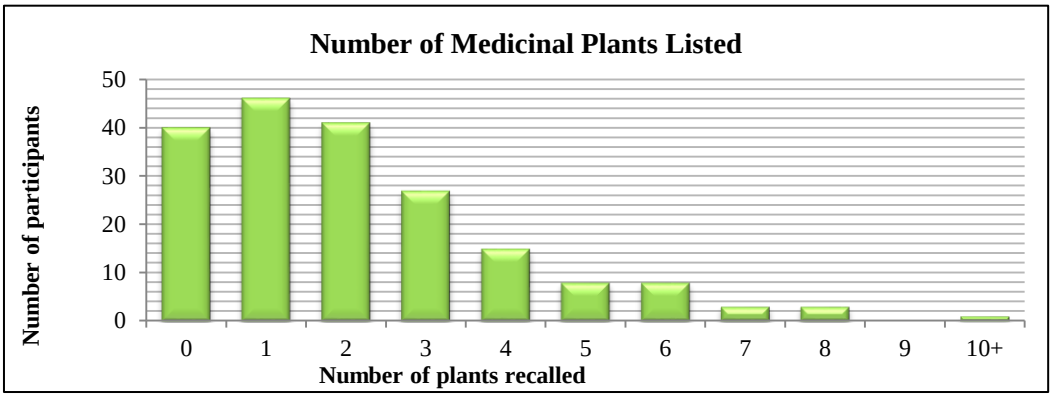


Figure 2-18 The Number of Medicinal Plants Recalled by Participants

Various fruits (e.g. *amla*, cranberry, dates, oranges, and figs), vegetables (e.g. broccoli, carrots, and karela), and herbs (e.g. basil, coriander, fenugreek, rosemary, and parsley) were documented. Other products such as *ajwain*, cannabis, cinnamon, chillies, curry leaves, dandelion, fennel, ginger, neem, nettles, rose, spinach, and turmeric were also cited by participants (Appendix 5). Despite having successfully named multiple herbal products in part A, 21% (n=30) of participants failed to name any plants with medicinal benefits in this section. This could have been because participants did not want to repeat themselves from part A as they had already listed numerous products; or because participants did not know of any medicinal plants, as suggested by participant 8 who listed nine herbal remedies in part A but said he could not name any plants. Some participants (2%, n=4) were able to recall more products when they were asked specifically about plants with medicinal benefits in comparison to section A of this question. The ways people use plants is dynamic (Heinrich et al., 2006); the range of plants recalled by participants illustrates that they had a considerable wealth of knowledge of medicinal plants (Appendix 5).

Question 9: ***Do you know any herbal remedies or products which can be used for the following conditions?***

In this question participants were presented with seven health conditions a mixture of minor and serious health problems (i.e. arthritis, cough, diabetes, headache, indigestion, itchy skin, and wounds) and asked to state any HMs they knew of which could be used to treat or manage the conditions. The rationale for selecting these conditions is because they are amongst some of the most popular health problems people seek treatment for in the UK (PAGB, 2009). Conditions such as cough, headache, indigestion and skin disorders are amongst the top ten ailments treated over the counter (OTC) and may be considered as minor health problems (PAGB, 2009; Pharmacy Research UK, 2014). Arthritis affects around 10 million people in the UK (NHS, 2014). Research into arthritis and ethnic minority groups in the UK has found that some forms of arthritis appear to be more common and more severe in SA ethnic groups (Samanta et al., 1992; Samanta et al., 2005). It is estimated that 3.3 million people in the UK have diabetes (Diabetes UK, 2015); SAs in the UK are up to six times more likely to have diabetes than the Caucasian population (Khunti et al., 2009). These health problems can have a serious impact on peoples' lives; despite this little is known about how the conditions are managed by SA populations (Khunti et al., 2009). By investigating participants knowledge of HMs used for these conditions a better understanding of alternative therapies used for these health problems can be assessed.

The majority of participants (86%, n=165) were able to list treatments for one or more of the conditions (Figure 2-19); just 8% (n=15) of participants listed a remedy for each of the seven conditions. Of the total population surveyed, 14% (n=27) were unable to state herbal remedies for any of the conditions listed. Figure 2-19 represents whether or not participants stated a response for each of the health conditions. It is evident that more participants were able to recall remedies for conditions such as a cough (n=151), indigestion (n=127), and wounds (n=98) in comparison to arthritis (n=65), diabetes (n=68), and headache (n=63). As cough, indigestion, and skin conditions are amongst the top ten conditions treated OTC in the UK (PAGB, 2009); this could explain why more participants knew of HMs to treat them in comparison to the other conditions.

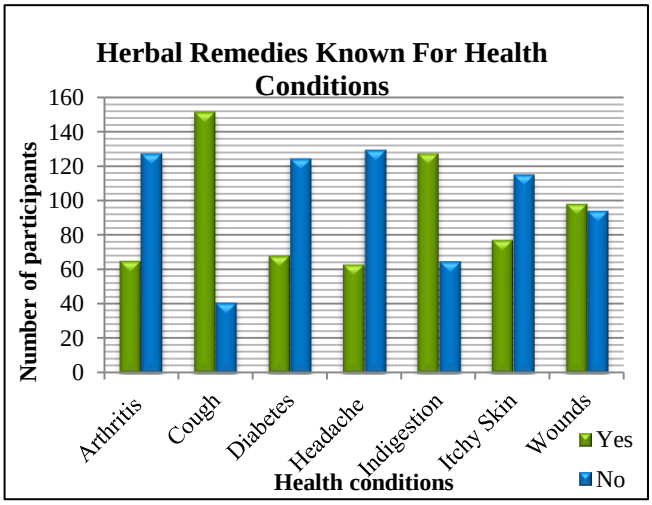


Figure 2-19 Herbal Remedies Known for the Health Conditions Listed

Figure 2-20 represents HMs stated by participants for each condition organised according to the age ranges - young, middle aged and older; it illustrates that for conditions such as itchy skin there was a similar number of responses from young, middle aged and older participants (n=25, 26, 26 respectively). For conditions such as cough and wounds there were more responses from younger participants than the other age groups. While, conditions such as arthritis and diabetes there were more responses from the middle aged and older participants in comparison to the younger participants. Although arthritis and diabetes can affect people of all ages (Arthritis Research UK, 2013a; Diabetes UK, 2015), middle aged and older participants appeared to have better knowledge of how to treat and manage them with HMs in comparison to younger participants.

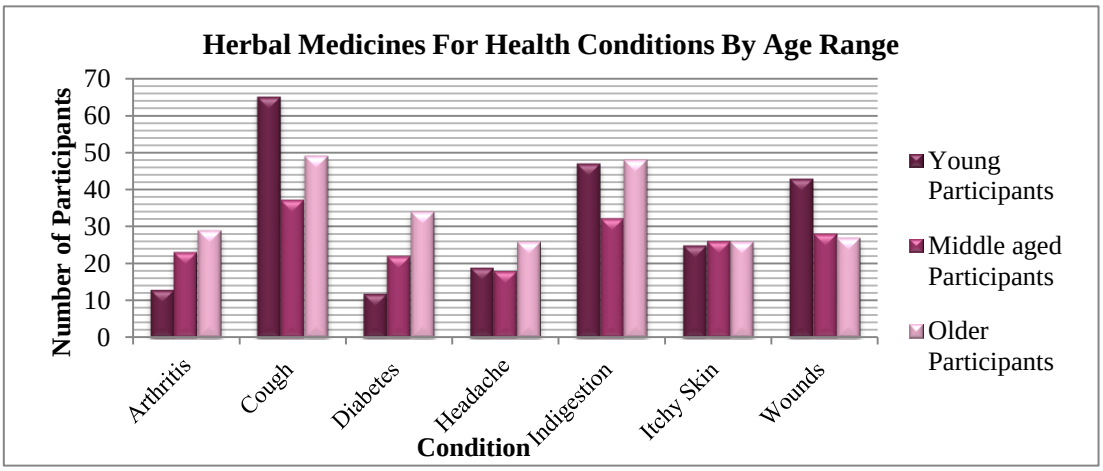


Figure 2-20 Herbal Medicines Cited for Each Condition by the Different Age Groups

Ginger was mentioned for its use in arthritis (n=9); due to its anti-inflammatory action which can help with the pain and loss of mobility caused by the condition (Grzanna et al., 2005). The notion that ginger is *garam* and thus improves circulation was frequently documented. It could be eaten in its raw form, soaked in water over night and the liquid taken in the morning, infused in tea, or incorporated into daily meals. Ginger powder, tablets, beverages, and many other formulations are now readily available and there is a vast amount of literature which supports the medicinal claims associated with its use (Polunin and Robbins, 1992; Pharmaceutical Press, 2013). Ali et al. (2008) say the anti-inflammatory effects of ginger have been known for centuries; although, evidence was only made available when research conducted identified the active components in ginger and claimed they worked like non-steroidal anti-inflammatory drugs (NSAIDs); moreover, ginger avoids the side-effects caused by NSAIDs. Ginger is known to contain a mixture of biologically active compounds which work in different ways to reduce inflammation; i.e. some compounds have an inhibitory effect on the production of inflammatory cytokines, while others inhibit the proliferation of inflammatory cells (Ali et al., 2008; Bartels et al., 2015). Fenugreek was

another popular remedy recommended for arthritis (n=10); the fresh or dried leaves could be incorporated in cooking or infused in tea, the seeds could be ground and blended with other herbs, or soaked overnight in water and drank the next morning. Massaging the affected joints with various oils was also recommended, including: black seed oil, flax seed oil, and garlic oil - made by infusing cloves of garlic in olive or mustard oil. There was a clear warning to avoid sour or acidic foods such as yoghurt, oranges, and lemons as they were believed to make the condition worse. Participant 62 claimed, *''lemon is very bad for arthritis, anything acidic should be avoided.''* However, there is no evidence to suggest cutting these substances out of the diet helps with arthritis; if anything they are an important source of nutrients so should be consumed as part of a balanced diet (Arthritis Research UK, 2013b).

For the treatment of coughs there were several common herbal remedies suggested; 50% (n=76) of participants listed honey as being useful to sooth a cough of which half (n=38) recommended taking it with lemon. While others suggested combining honey with black pepper, ginger, brandy, cinnamon, or turmeric. Honey is made up of a mixture of compounds which give it anti-inflammatory, anti-bacterial, and antioxidant effects which explain why it has been used in traditional medicine for coughs (Oduwole et al., 2014). Liquorish, mint, tulsi, fennel, black seed, *banaksha,* and salt water gargles were also listed as being used for a cough. *Banaksha* (*Viola odorata* L., sweet violet) has traditionally been used to treat cough, catarrh, fever, headache and pain, hence its popularity for relieving the symptoms of coughs and colds (Bhatia et al., 2014).

For diabetes it was well known that there was no cure for the condition but blood glucose (BG) levels could be managed and kept in control by the consumption of HMs. Of the 68 participants who listed a herbal remedy for diabetes, 56% (n=38) mentioned the hypoglycaemic properties of karela (*Momordica charantia* L.). Participant 107 claimed, *''karela is good for diabetes as bitter things can be used to counteract the sugar.''* Fresh karelas are widely available in the UK and can be consumed as a curry, juiced, or soaked overnight in water. Various commercial karela formulations are also available, including: dry powder, concentrated juice, and tablets. There is an abundance of research conducted on the use of karela in diabetes around the world (Baxter, 2008; Pandey et al., 2011; Lone et al., 2012). There are several proposed methods which can explain the hypoglycaemic effects of karela; it is believed to reduce glucose absorption, increase glucose uptake in liver cells, and improve insulin secretion (Jayasooriya et al., 2000). Fenugreek (*Trigonella foenum-graecum* L.) was mentioned by 16% (n=11) of participants who highlighted the anti-diabetic properties were best obtained by soaking the seeds in water over night, or consuming the leaves regularly as part of the diet. Studies have shown that fenugreek can stimulate glucose

uptake into cells and plays a part in activating the insulin secretion signalling pathway; thus, helping reduce BG levels and improving insulin response in diabetics (Vijaykumar et al., 2005; Chevallier, 2007). Other products described by participants for diabetes included: tulsi infused in water or tea, turmeric with milk, ginger mixed with cinnamon and honey, honey to substitute other sugars, figs soaked in milk overnight, gram flour chapattis instead of wheat flour, okra as a curry or soaked in water over night, *amla*, cinnamon, chick peas, curry leaves, olives, grapefruit, and neem.

Just 63 participants (33%) said they knew of HMs which could be used to treat a headache. The first response from many participants was often 'paracetamol' but participants were reminded that this research was focused on HMs, so these responses were disregarded. Herbal remedies to treat a headache included: massage the head with oil (olive, almond, coconut, or mustard oil), drink plenty of water, masala tea, mint tea, ginger tea, ginger infused in water, holy water, honey in hot water, *ajwain* seeds chewed or infused in water or tea, millet flour compress on forehead, menthol rubbed onto forehead, steam inhalation with menthol crystals, cloves warmed and the fumes released inhaled, and black pepper chewed or infused in tea. Participant 85 claimed, *''you do not get headache if your digestion is ok.''* This point was also highlighted by several other participants and an Ayurvedic practitioner (Dr. Pankanj Chansarkar, Kerala), who described the anatomy and explained the causes of a headache. Dr. Chansarkar was introduced to the researcher during a field trip to Kerala in April 2014, where he explained how according to Ayurveda the build-up of gasses (digestive impurities) in the alimentary canal feedback and signal pain receptors which are recognised as a headache. According to Dr. Chansarkar a headache is a sign that there is insufficient clearing of the digestive tract. He recommended a digestive aid (*Triphala*- a mixture of *amla, beleric and harad*) to help clear the gastro-intestinal tract in order to eliminate the headache. *Ajwain* is a bitter tasting, aromatic seed that is rich in essential oils which contain thymol. It is commonly used in cooking to add flavour and has numerous health benefits; it is a popular digestive aid which can help with gastro-intestinal ailments (i.e. flatulence, diarrhoea, and indigestion) and provides anti-spasmodic, laxative, and pain relief effects (Bairwa et al., 2012). It appears many of the research participants in this study who treated headaches with a digestive aid were aware of this traditional knowledge which is not well documented.

Indigestion is a common ailment which causes a discomfort in the upper abdomen, often associated with a bloated sensation, nausea and excessive belching. It was uncovered that SA communities in this research used the term 'gas' to describe these symptoms. The pathophysiology is the same; participants had a clear understanding of what indigestion was and how to treat it. Indigestion may be experienced due to eating 'heavy' meals (large portions, high fat or fried foods), stress, medication, or because of poor metabolism. From

the results (Figure 2-19) it is clearly a condition many of the participants have knowledge of how to treat; 66% of participants (n=127) surveyed stated one or more herbal remedies to treat indigestion. Remedies including: *ajwain*, black salt, cardamom, fennel, lemon water, mint, milk, *hing*, and *churan* were recommended for treating indigestion. *Churan* (also known as *churna*) is a digestive powder composed of several ingredients including ginger, cumin, *ajwain*, rock salt, black pepper, fenugreek, hing, and fennel; although, different manufacturers may have their own recipes (Chaudhury and Rafei, 2001).

There are a variety of commercial products available for itchy skin which are based on HMs. Most of the products recommended by participants for itchy skin were topical preparations such as the sap from the aloe vera plant rubbed onto the skin (mentioned by 35% of respondents, n=27), oils (almond, coconut, mustard, and olive oil) and pastes (turmeric with yoghurt or olive oil, crushed garlic, onion juice, or a mixture of honey and cinnamon). A large number of participants (40%, n=31) suggested using an oil to stop the itching as it would moisturise the skin and reduce the dryness, which makes the itch-scratch cycle worse (Yosipovitch and Hundley, 2004). Other remedies were formulated to be ingested to provide systemic relief from the itching such as: turmeric in milk several times a week, dried neem leaves mixed with honey to make small balls taken daily, neem leaves infused in warm water or tea.

The final condition participants were asked to detail a herbal remedy for was wounds; 98 participants (51%) stated a remedy they knew of which could be used for wounds. A considerable number of participants (77%, n=75) listed the benefits of turmeric for aiding wound healing. There were several methods of preparation and administration noted. Some participants claimed turmeric should be made in to a drink by warming with milk and drinking it every night; some recommended adding black pepper, clarified butter (*ghee*) or olive oil. While others preferred to use a topical formulation to give a local effect by making a paste with oil (e.g. olive oil or mustard oil), *ghee*, or water. The medicinal value of turmeric is undergoing extensive investigation. It has antimicrobial and anti-inflammatory action which may explain its traditional use in wound healing. The antimicrobial function prevents bacterial proliferation when the skins defences have been breached; while the anti-inflammatory action will help the wound heal quicker (Colalto, 2010). Various other topical and internal preparations were also recommended for wounds including: the use of neem, honey, aloe vera and some even suggested rubbing salt directly into a wound to stop it bleeding.

A list of the herbal remedies documented by participants can be found in Appendix 5.

Participants' Knowledge of Herbal Medicines

Participants' knowledge of HMs was measured by calculating the total number of 'items' recalled; this included any ingredients, products or remedies participants mentioned throughout the entire survey. Each response was given a value of one and this was added up to give the total number of items recalled, if an item was mentioned several times it was only counted once.

Figure 2-21 illustrates the total number of items recalled increased as more questions were asked in comparison to Figure 2-17, which just looked at participants responses from question 8A. The proportion of participants who did not state any herbal products reduced from 7% (n=13) to 5% (n=10); while, those who recalled twenty or more items increased from 3% (n= 6) to 10% (n=19). The reason for an increased number of items recalled could be due to the prompts given, i.e. asking participants' to identify medicinal plants or products for specific health conditions (Harris and Brown, 2010); or even because participants had more time to think about herbal remedies as the questionnaire progressed. Question eight alone would not be sufficient to judge the participants knowledge of HMs; but, by having additional sections and questions a better indication of knowledge can be assessed.

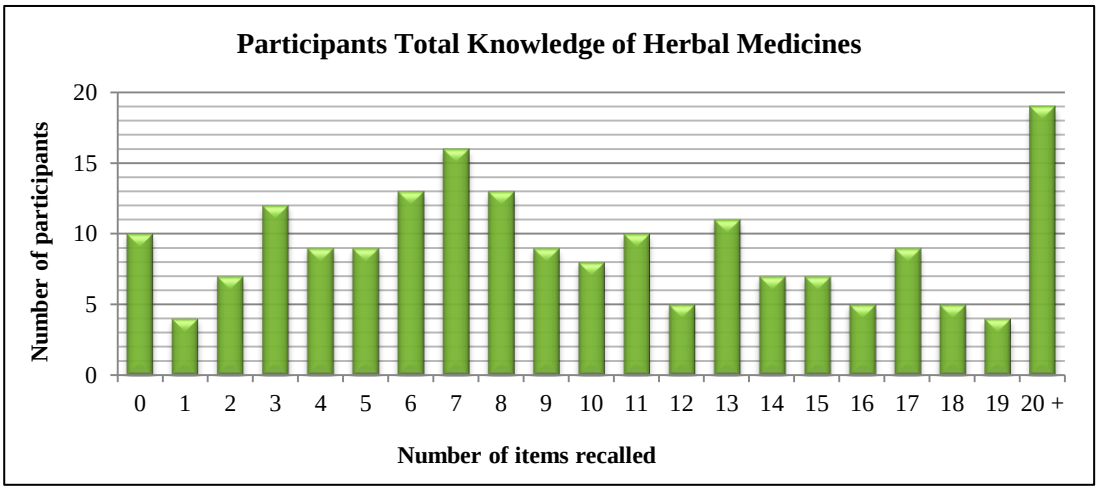


Figure 2-21 SATMED Participants' Total Knowledge of Herbal Medicines

The results demonstrate that there was a great wealth of knowledge of HMs amongst the research sample. There are various parametric techniques which can be used to explore the relationships between variables in this data. For example, a t-test can be used when there are two defined groups in a variable; i.e. it can be used to explore the relationship between the difference in knowledge across male and female participants. While analysis of variance (ANOVA) techniques can be employed when there are two or more groups within a variable; i.e. to determine the difference in knowledge across the different age groups (Pallant, 2007).

Independent sample t-test

An independent sample t-test can be used to determine if there is a statistically significant difference between two variables. It can be used to explore the differences in the mean number of responses between male and female respondents. Table 2-3 identifies the number of male and female participants (N), and summarises the mean (M) number of items recalled for each group, along with the standard deviation (SD). The mean number of responses per female participant was 11 (SD = 7.01), and for male participants M = 7 (SD = 6.18). From this it can be concluded that the male participants recalled fewer items but there was less variance in their results.

Table 2-3 T-test Group Statistics

Gender	N	Mean number or responses	Standard Deviation
Male	60	7	6.18
Female	132	11	7.01

Table 2-4 Independent Samples T-test

Independent Samples Test									
	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Standard Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Equal variances assumed	2.297	0.131	3.386	190	0.001	3.565	1.053	1.488	5.642
Equal variances not assumed			3.550	128	0.001	3.565	1.004	1.578	5.552

The first part of Table 2-4 indicates the 'Levene's Test for Equality of Variances' which tests whether the variation for the two groups is the same. If the significance probability (Sig) value (P) is greater than 0.05 then results from 'equal variances assumed' should be used; whereas a lower Sig value indicates results from 'equal variances not assumed' should be used. In this instance the Sig value is 0.131, as this is larger than the standard of 0.05, equal variances can be assumed (Pallant, 2007). The Sig. (2-tailed) result is then used to see if there is a significant difference between the two groups (i.e. male and female participants), if this value is equal to or less than 0.05 it suggests there is a significant difference between the means of the two genders, whereas a higher value would suggest there is no significant difference. Table 2-4 shows that the Sig (2-tailed) value p = 0.001, this is lower than the

standard 0.05; therefore, this indicates there is a significant difference between the mean number of items recalled by male and female participants (Pallant, 2007; Petrovics, 2012).

Effect size statistics can then be used to provide an insight into the strength of the association to show that the results have not just occurred due to chance, as well as highlighting the statistical significance (Pallant, 2007; Petrovics, 2012). Eta squared is one way of calculating the effect size statistic. The eta squared value can range from 0 to 1 and can be interpreted in the following way: 0.01 is a small effect, 0.06 is a moderate effect, and 0.14 is a large effect (Cohen, 1988). The following formula is used to calculate eta squared:

$$\text{Eta squared} = \frac{t^2}{t^2 + (N12 + N2 - 2)}$$

$$\text{Eta squared} = \frac{3.386^2}{3.386^2 + (60 + 132 - 2)} = 0.06$$

The eta squared value of 0.06 suggests there is a moderate association, that this is a significant finding and not just because of chance. In summary, the results from of the t-test and eta square confirm there is a difference in knowledge between the two genders; female participants had more knowledge of HMs in comparison to male participants. This is supported by the Mintel Report (2009), which claims women have more knowledge of HMs, thus they use them more than men. Furthermore, Ernst (2000) also states that women of all ages use HMs more frequently than men. Male participants in this investigation claimed they did not take any HMs for their health (in question 1), but later revealed they did take HMs given to them by their grandparents, mother or wife (question 6). This supports the nurturing role females have been associated with throughout history (Ehrenreich and English, 1973; Hegg, 2013). The traditional role for SA women was as the house keeper; which involved cooking and looking after their husband and children once they started their own families (Samanta, 1999; Chaudhury and Rafei, 2001). As women were the ones who have traditionally been the main cooks in the home they were best equipped with the knowledge of how to use food as medicines (Jennings, 2014). This nursing and caring role women have been traditionally associated with is evidently still common amongst SA communities in the UK; where female participants appear to have more knowledge of HMs than males.

Analysis of Variance

An Analysis of Variance (ANOVA) tests the hypothesis that the means of two or more variables are equal (Minitab Inc, 2014). It compares the variance between the groups, enabling conclusions to be made regarding variations among results (Pallant, 2007). A one-way ANOVA can be used to compare the variance between groups in an independent variable; for example; how knowledge (dependent variable) varies with age (independent variable). The Null hypothesis would be that knowledge is equal despite age; while the Alternative hypothesis would be that there is a difference in knowledge between the age groups. Table 2-5 summarises the mean number of responses given by each age group, with the standard deviation, standard error, and 95% confidence intervals for the mean.

Table 2-5 ANOVA Summary of Participants Age and Mean Number of Herbal Medicines Recalled

Age	N	Mean	Standard Deviation	Standard Error	95% Confidence Interval for Mean	
					Lower Bound	Upper Bound
20 or under	43	6	6.121	0.933	4.77	8.54
21 - 30 years	47	8	5.207	0.760	6.88	9.93
31 - 40 years	10	12	5.633	1.781	8.77	16.83
41 - 50 years	12	13	11.156	3.220	6.50	20.67
51 - 60 years	21	13	7.423	1.620	9.62	16.38
61 years +	59	12	6.346	0.826	10.55	13.86
Total	192	10	6.945	0.501	9.25	11.22

Table 2-5 identifies that the mean number of responses increases with age. A potential reason for the slightly lower mean for the 61+ age group is because during data collection it was noted that some of the older participants were reluctant to share their knowledge or take HMs at the same time as taking Western medicines. As reflected by participant 125, '*I do not want to tell anyone about any herbal remedies as if something goes wrong people will blame me. I have so much knowledge about herbal remedies but I am scared to share it, in this country people do not want to know*!' In addition, many of the participants in the 61+ age group took prescription medicines for various health conditions such as diabetes and cardiovascular disease, and commented on how they had stopped using HMs. This was mentioned by several participants including participant 112 who claimed, *'I do not take any herbal remedies, I only take the doctors medicines because I have to,*' and participant 115 who said, *''I do not really use much herbal remedies now, I just take my prescription medicines.''*

Table 2-6 ANOVA Summary of Variance between Participants Age and Number of Responses

	Sum of Squares	Degrees of freedom	Mean Square	F	Sig.
Between Groups	1299.291	5	259.858	6.108	0.00003
Within Groups	7913.163	186	42.544		
Total	9212.453	191	-		

Table 2-6 represents the results of the ANOVA test which shows the sum of squares, which is a measure of variation or deviation from the mean, and degrees of freedom (Minitab Inc, 2014). If the Sig value is less than or equal to 0.05 there is a significant difference among the mean scores of the dependent variable. The sig value indicated in Table 2-6 is 0.00003; consequently this shows there is a significant difference in the mean results.

The F test represents the variance between the groups divided by the variance within each group. A significant F ratio would suggest there is more variability between the groups, due to the independent variable (i.e. the total knowledge) than there is within the group. If the F value is around 1 we can assume the null hypothesis is correct (Pallant, 2007). Table 2-6 identified that the F value is 6.108 thus the null hypothesis, that there are no significant differences in number of responses between the different age groups, needs to be rejected; while, the Alternative hypothesis, that there is a difference in knowledge between the age groups, can be accepted.

To calculate the effect size the following formula can be used:

$$\text{Eta squared} = \frac{\text{Sum of squares between groups}}{\text{Total sum of squares}} = \frac{1299.291}{9212.453} = 0.14$$

The one-way between-groups ANOVA was conducted to explore the impact of age on total knowledge (i.e. the number of herbal remedies participants recalled). The results show there was a statistically significant difference as $p < 0.05$ and F test results show: $p = 0.0003$, $F = 6.108$. The effect size calculated using Eta squared, was 0.14 indicating a large effect; reinforcing a statistically significant result, that knowledge was different across the various age groups (Pallant, 2007). The results of the ANOVA test suggest there is a difference in knowledge of HMs amongst the different age groups. Although, the knowledge of HMs is transmitted to younger SA generations in the UK, as exemplified by their responses throughout this investigation, the older participants still had more knowledge of HMs. This is supported by Pieroni et al. (2010) who also found that middle-aged and older Bangladeshi

participants in their research had better knowledge of traditional HMs than younger participants.

2.6.3 The Use of Prescription Medication

This section of the questionnaire set out to investigate whether or not participants took prescribed CWM alongside HMs. Participants were asked what CWM they were prescribed by their doctor, nurse, pharmacist or other HCPs, to explore the safety of consuming HMs, and the potential interactions between HMs and CWM. Participants were asked whether or not they shared information about their use of HMs with their HCPs, to gain insight into the relationship between SA patients and their HCPs. In addition, the side-effects reported from CWM and HMs were compared.

Question 10 A: ***Do you take any regular prescribed medicines?***

10 B: ***If Yes, and you are happy to say please state what medicines you take.***

All of the research participants (n=192) were asked whether or not they took any regular prescribed medicines; of which more than half of the population (55%, n=106) stated they did. This included the treatment for a range of health problems including: pain relief, asthma, diabetes, cardiovascular disease, topical treatments for skin conditions, and vitamins to supplement deficiencies (Table 2-7). The 45% (n=86) of participants who said they did not take any prescription medication were asked to move on to question 13 as the following questions were specifically for those who did take prescription medication.

The following questions in this section represent the responses for 106 participants who took prescription medication.

Figure 2-22 illustrates the age profile of participants who took regular prescribed medication; it identifies that more than half (n=57) of the 106 participants who took prescribed medication were in the 61 years plus age group. Despite this, participants from all of the other age groups also took prescription medicines. Participants were asked to list their medication, if they did not want to disclose this information they were asked to move on to the next question. Some of the older participants or

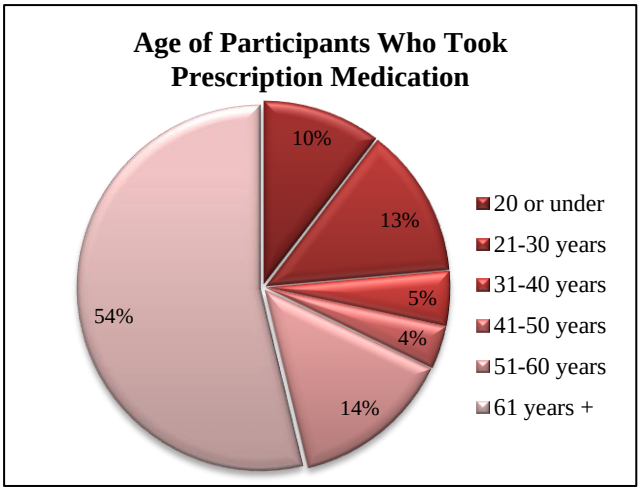


Figure 2-22 Age Profile of Participants Who Took Regular Prescribed Medication

those who did not speak fluent English, could not name the specific medicines, but knew the conditions they were being treated for. Table 2-7 summarises the medical conditions and medicines listed by participants. The results revealed that participants were taking medication for a vast range of health conditions; the most common conditions mentioned were hypertension (n=47), diabetes (n=36), and high cholesterol (n=25). Other common health problems included thyroid disorders, asthma, and allergies. The types of CWM taken can be categorised by age groups; medicines such as the contraceptive pill, acne treatments, and iron supplements were common in younger females (aged less than 20 to 30 years). The few young males who said they took regular prescribed medication used inhalers for asthma. Medicines for arthritis, hypertension, diabetes, and other chronic conditions were generally mentioned by the latter half of the middle aged group (51-60 years) and elderly age group (61 years plus). Vitamins, topical preparations for skin conditions, thyroid disorder medication, and asthma treatments were mentioned by both genders and all age ranges.

Table 2-7 Summary of Prescription Medicines Taken by Participants

Medical Conditions:	Conventional Western Medicines Taken:
Cardiovascular disorders e.g. Angina, Hypertension, & Hypercholesterolemia	Aspirin, Clopidogrel, Warfarin, Furosemide, Spironolactone, Amlodipine, Felodipine, Bendroflumethiazide, Ramapril, Losartan, Telmisartan, Atenolol, Bisoprolol, Propranolol, Atorvastatin, Simvastatin, Rosuvastatin, Ezetimibe.
Diabetes	Metformin, Gliclazide, Pioglitazone, Insulin injections, Byetta injection.
Epilepsy	Keppra – Lamotrigine.
Gastro-intestinal disorders	Lansoprazole, Omeprazole, Mebeverine, Senna.
Pain relief	Paracetamol, Gabapentin, Codeine, Co-dydramol, Morphine, Ibuprofen, Naproxen.
Pulmonary disorders e.g. Asthma & emphysema	Steroid inhalers (seretide and clenil), Salbutamol, Tiotropium, Montelukast tablets.
Anti-depressants & mood stabilisers	Amitriptyline, Citalopram, Fluoxetine, Mirtazipine, Venlaflaxine.
Skin conditions	Duac acne gel, Creams for eczema (steroid and emollients).
Thyroid disorders	Carbimazole, Levothyroxine (thyroxine).
Miscellaneous	Anti-histamines (Cetirizine, Loratadine), Contraceptive pills, Methotrexate, Pyridoxamine, Quinine, Vitamins, Folic acid.

The simultaneous use of HMs and CWM can have serious consequences; by asking participants what HMs and prescription medicines they took, the information could be used to identify any potential interactions. At the time of conducting the surveys the researcher

did not intend on identifying interactions and making participants aware; there was a disclaimer to inform participants to speak to their HCPs if they had any queries about their medication, which the DMU ethics committee advised the researcher to include. By looking at the list of CWM and HMs participants consumed, a range of adverse effects and potential interactions can be identified.

Fugh-Berman (2000) says, *''concurrent use of herbs may mimic, magnify, or oppose the effect of drugs.''* Williamson et al. (2013) identifies an interaction when the effect of a drug is changed by the presence of another substance; this includes herbal medicines and food. HMs can alter the drugs pharmacokinetics by affecting the drugs absorption, distribution, metabolism and elimination. Cytochrome (CYP) P450 are a group of enzymes which metabolise a variety of substrates and have an important role in drug metabolism; the rate of enzyme activity can be affected by numerous herbal products. Some products may increase the rate of metabolism (enzyme inducers), rendering the drug ineffective at sub-therapeutic levels if metabolised too fast; alternatively if the drug is metabolised slower than anticipated, it can accumulate and cause toxicity. Tovar and Petzel (2009) claim, *''Garlic is safe when used in dietary amounts''*; however, consumption at higher doses and stronger concentrations for medicinal purposes may be associated with underlying risks especially if used simultaneously with other drugs. Garlic has an enzyme inducing effect; therefore, it can reduce the effectiveness of drugs metabolised by CYP 3A4 (Spurgeon, 2000; Foster et al., 2001). Ponnusankar et al. (2011) investigated the effects of *Triphala*, a popular Ayurvedic mixture with multiple health benefits, on the CYP 450 enzyme; they found the three components of *Triphala (amla, beleric and harad)* caused inhibition of the enzyme; thus, reducing drug metabolism.

Other interactions can be caused by HMs if they affect the drugs pharmacodynamics by imitating or inhibiting a drugs mode of action (Chavez et al., 2006). One of the most frequently mentioned herbal ingredients was ginger, cited 179 times during the surveys. Its use as an anti-emetic, digestive aid, anti-inflammatory, analgesic, and for coughs and colds was commonly identified by participants. Ginger can have hypoglycaemic effects; hence, if consumed with other anti-diabetic medicines the risks associated with low blood glucose (BG) levels may be fatal (Newall et al., 1996). Fenugreek, karela, garlic, turmeric, tulsi and aloe vera were also identified by participants as being used in diabetes to lower BG. Researchers who have explored the hypoglycaemic properties of fenugreek have found it to be a very powerful anti-diabetic agent due to the actives: gonelline, nicotinic acid and coumarin found in the defatted portion of the seed (Pandey et al., 2011). A significant reduction in BG levels caused by taking HMs could lead to poor diabetic control, interactions with medication, and serious health risks for the patient (Sethi et al., 2004).

Cloves have been traditionally used as digestive aids and for toothache, due to their carminative and analgesic effects. With a high eugonel content, which inhibits platelet aggregation, interactions between conventional anti-platelets (aspirin and clopidogrel) and anti-coagulants (warfarin) have been identified (Pharmaceutical Press, 2013). This is also true for many other herbals including: garlic, ginger, *hing*, fenugreek and turmeric which can increase blood clotting time (Newall et al., 1996; Spurgeon, 2000). People are at an increased risk of haemorrhage due to the dual blood thinning effect of combining CWM with HMs (Heck et al., 2005).

Liquorice was often used by participants to treat coughs and upper respiratory tract congestion. Large quantities and long term ingestion has been associated with hypokalaemia and hypertension (Lin et al., 2003; Chevallier, 2007). Due to its stimulant and fluid retention properties liquorice has been known to increase blood pressure, rendering anti-hypertensive medicines less effective (Williamson et al., 2013). If taken with prescription diuretics (e.g. Bendroflumethiazide or Furosemide), which are known to be associated with hypokalaemia, potassium levels may fall below optimum levels. Hypokalaemia is usually asymptomatic so people may not be aware of the consequences; however, sometimes it may present as symptoms of weakness, muscle cramp, constipation and palpitations (Lederer and Batuman, 2014). Without the correct knowledge, this may not be identified or treated appropriately.

The incidence of interaction with CWM and HMs is not fully known; Williamson et al. (2013) highlight that despite this many HCPs openly admit they lack knowledge in this area. The insufficient reporting of interactions and adverse effects may be due to inadequate training or a lack of knowledge of how to report them. Interactions between HMs and CWM may exist; but, if people do not tell their doctors and pharmacists they will not be able to intervene. Alternatively, people may not be aware there is an interaction or adverse effect as they do not always present with symptoms.

Question 11: ***Do you tell your (a) doctor and (b) pharmacist what herbal remedies you use?***

Figure 2-23 illustrates the responses of whether or not the participants who took regular prescribed medication told their doctor and/or pharmacist about their use of HMs. Of the 106 participants who did take regular prescribed medication, 13% of participants (n=14) did not consume any HMs, so they answered '*not applicable*'. Just 18% of participants (n=19) said they did tell their doctor, and only 5% (n=5) told their pharmacist about herbal remedies they took alongside their prescription medicines. A significant proportion of participants (69%, n=73) revealed they did not tell their doctor and even more (82%, n=87) declared they did not tell their pharmacist. Similar findings were illustrated by a study conducted by Bhopal (1986a), who found participants did not tell their doctor about herbal remedies as they felt it was a separate matter. Results from the Ipsos MORI report (2008) revealed that 20% of respondents in their survey did not think there was any need to inform their general practitioner (GP) about HMs they used; while 56% felt it was important to tell their GP. Table 2-8 summarises the reasons why participants in this study did not tell their doctor or pharmacist about their use of HMs.

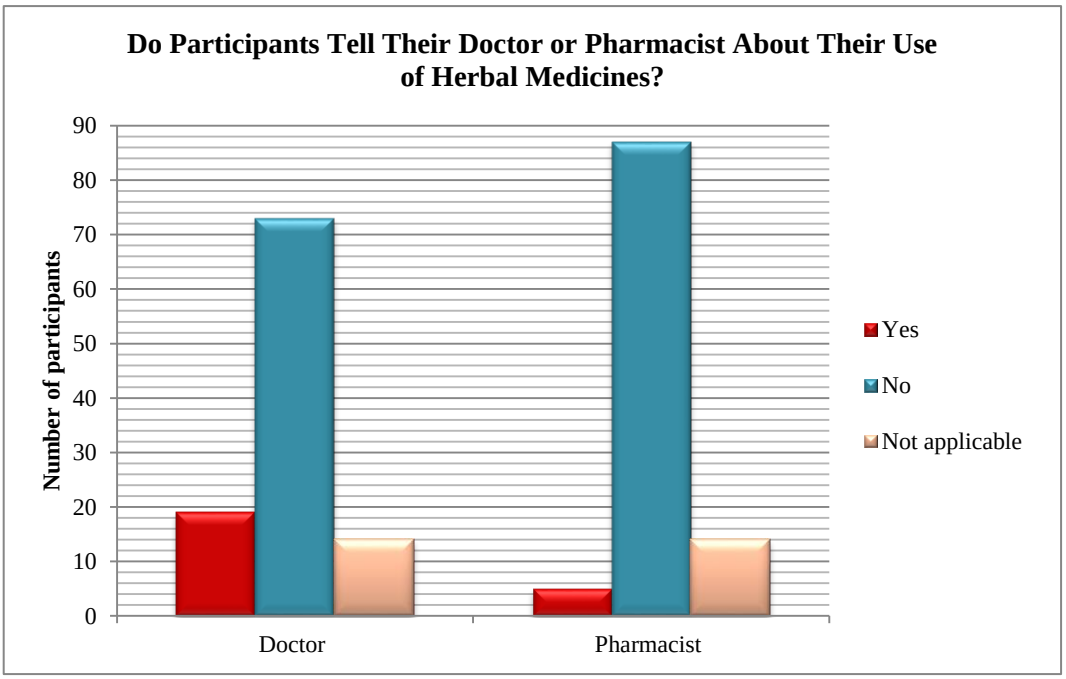


Figure 2-23 Do Participants Tell Their Doctor or Pharmacist about Their Use of Herbal Medicines?

Table 2-8 Participants Reasons for Not Telling Healthcare Professionals about Their Personal Use of Herbal Medicines

Participant Number:	Reasons For Not Telling Healthcare Professionals About Personal Use of Herbal Medicines:
67	*''I do not tell the doctor about herbal medicines as doctors do not believe you if you tell them they are good.''*
73	*''I do not tell the doctor or pharmacist about herbal remedies as the doctor says why are you coming to us if you want to try different medicines.''*
77	*''I do not tell the doctor about herbal remedies taken as he is not bothered.''*
105	*''My doctor has said she would not advise mixing herbal medicines.''*
109	*''I do not tell my pharmacist as I never see one.''*
111	*"I do not tell the GP about herbal medicines I take as he might say do not take it."*
113	*''The doctors do not believe in herbal remedies so why should I tell them what I use!''*
117	*''Doctor refuses the use of herbal medicines and says do not take them without our guidance.''*
124	*''Too scared to tell the doctor about any herbal medicines taken.''*
138	*''I rarely get to see my GP and do not ever see or speak to a pharmacist when I go to collect my medicines.''*
156	*''I do not tell my doctor or pharmacist about herbal medicines used as they do not recommend it.''*

Several crucial reasons for not telling the doctor or pharmacist about personal use of HMs were identified. From the participants' responses (Table 2-8) it appears that participants thought HCPs lacked interest in HMs and were not concerned if they were consumed, as stated by participants 67, 77, and 113. If HCPs are not interested in their patients' use of HMs, then patients will feel reluctant to share this information with them (Staines, 2011). HCPs need to be broad-minded and approachable so that patients feel comfortable talking to them about their use of HMs (Newall et al., 1996; Vickers, 2000). Participants in this study claimed they would be advised to stop using HMs if they told their HCPs, as exemplified by the comments from participants 73, 117, and 156. An example of how taking HMs can affect patients' compliance with CWM and why HCPs may tell patients not to take HMs was demonstrated by Vohra (2015) who detailed the conversation between a pharmacist and a SA patient during a Medicines Use Review (MUR). During the MUR the patient explained to the pharmacist that he wanted to stop taking his antidepressant as he was taking a traditional Ayurvedic remedy instead; the pharmacist said, '*Conventional medicine has been clinically trialled and is proven to be effective; Ayurvedic preparations are unstandardised and have not gone through rigorous patient trials. They also have the potential to interact and potentially reduce the effectiveness of prescribed medicines.''* There are several reasons why

HCPs may tell their patients to stop taking HMs including: the risk of herb-drug interactions, adverse effects, reduced compliance with conventional therapy if patients take HMs, and most importantly the lack of scientific evidence to support the safety and efficacy of HMs (Fugh-Berman and Ernst, 2001; Edwards et al., 2012); this concept has been explored further in chapter 3.

Evidently there was a fear of telling HCPs about HMs consumed as they may be regarded as inferior to CWM; as exemplified by participant 124 who said she was, *''Too scared to tell the doctor about any herbal medicines taken.''* Also participant 39 claimed, *"My son is a doctor and will not let me take anything herbal, as he says they are all rubbish, even if I know it is good for me."* Dash and Kashyap (1980) documented that traditional medicine was considered to be substandard in comparison to Western medicine. Participants in this study revealed their anxiety of being treated differently or not treated at all if they exposed their use of HMs to their HCPs. While conducting the questionnaire with participant 2 she said, *"The doctor might refuse my treatment if he knew I was taking herbal medicines!"* Similarly participant 73 said, *"The doctor says why are you coming to us if you want to try different medicines."* Language and cultural barriers which prevented participants from sharing information about their use of HMs with the HCPs were also identified by participants. Participant 122 was diagnosed with end stage liver cancer, and she revealed how the terrible effects of chemotherapy were relieved by the consumption of HMs, but she did not tell her consultants as she could not speak any English. Some concepts may be difficult to explain to Western HCPs; for example, there is no translation for *bhye* in English and so it is very difficult to explain this condition to Western HCPs. In turn, this may make people feel like HCPs do not understand their healthcare requirements. This was also reported by participants in the study conducted by Pieroni et al. (2010), which revealed that communication problems between Bangladeshis and HCPs in the UK prevented patients from using CWM appropriately.

An interesting point raised by participants 109 and 138 was that they hardly saw their doctor or pharmacist and that is why they did not tell them about their use of HMs; they went on to say if they did see their HCPs, they would mention it. Participants mentioned that they got their prescriptions delivered so did not have any contact with their doctor or pharmacist. The increased popularity of repeat prescription services which are designed to save time for the patient, doctors surgeries, and pharmacies may not be entirely beneficial to patients if it means they do not get to see their HCPs. Ultimately, healthcare is a complex issue especially when combining different cultures and traditions. The results from this question have provided clear examples of why people do not tell their HCPs about the simultaneous use of HMs with CWM. Newall et al., (1996) claim that HMs are becoming more popular and

therefore HCPs should be aware of alternative therapies; in addition, patients should feel comfortable speaking to their HCPs about their use of HMs without feeling like they are being judged.

Question 12 A: ***Do you notice any unwanted effects from your prescription medicines?***

12 B: ***If Yes, what do you normally do to relieve the symptoms?***

From the 106 participants who took prescription medication, 43% (n=46) disclosed they had experienced unwanted effects from their medicines (Figure 2-24). Participant 85 claimed, *''Doctors tablets always give me side-effects.''* This was supported by participant 87 who said, *''When you take Western medicines expect more side-effects and tablets to follow.''* Effects such as nausea, diarrhoea, indigestion, cramp, insomnia and back pain were reported by participants; more serious problems such as diabetes and hypertension induced by medication were also noted (Table 2-9). For the relief of the side-effects the majority of participants (54%, n=57) revealed they would go back to see their doctor. Participant 29 stated, *''For side-effects from a tablet the doctor just gives another tablet.''* 31% (n=33) of participants who experienced unwanted effects said they would do nothing, while 15% (n=16) would try a herbal remedy. There was some mention of CWM being *'garam'* thus causing many of the side-effects associated.

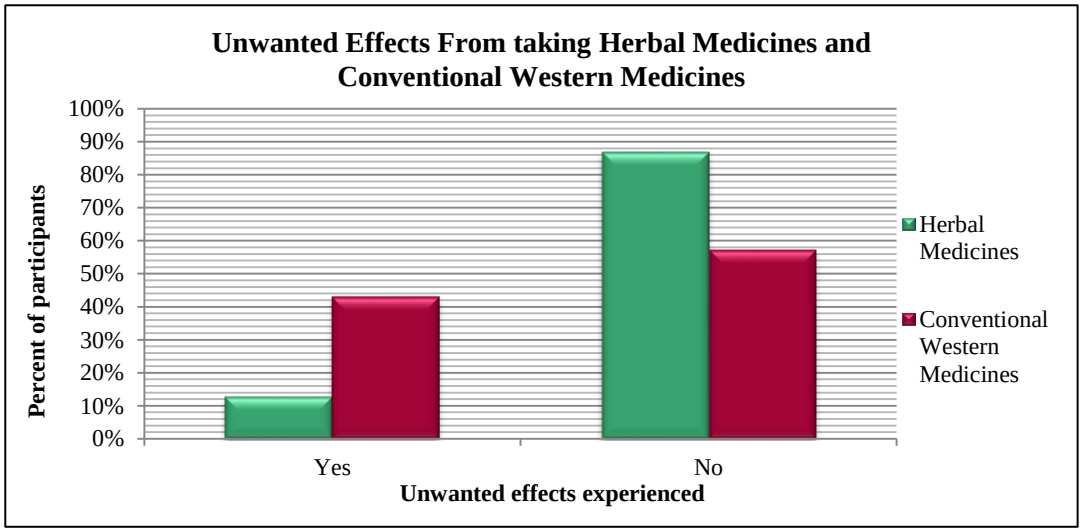


Figure 2-24 Unwanted Effects Experienced from Taking Herbal Medicines and Conventional Western Medicines

Participants were asked if they experienced any unwanted effects from their prescription medicines to compare the results to a previous question which asked if they experienced any unwanted effects from the consumption of HMs. Figure 2-24 shows a higher proportion of participants said they experienced side-effects from CWM (43%, n=46), than from HMs

(13%, n=16). A survey by Abbot et al. (1996) on users of HMs found that just 8% (n=32) of participants said they experienced adverse effects, and none of the effects were serious. Similarly the participants in this study who said they experienced unwanted effected from HMs, most of the effects reported were minor (Question 4, Table 2-2). In contrast, more serious side-effects caused by CWM were noted, such as: palpitations, cramps, nerve damage, and thiazide induced hyperglycaemia leading to diabetes (Table 2-9). Some of the side-effects reported for HMs and CWM were similar, both were associated with the concept of being '*garam'* (i.e. warming the blood and acting as a stimulant); consequently, side-effects such as indigestion and nose bleeds were reported. Generally, fewer side-effects were reported by participants taking HMs; the belief that HMs are natural and safe, so do not have any side-effects was noted by participants including participant 85 who claimed*, ''Herbal medicines have less side effects and are safer''* similar views have been documented in numerous other studies too (Ipsos MORI, 2008; Mintel, 2009; Ravindran et al., 2009).

Table 2-9 Side Effects from Prescription Medicines

Participant Number:	Side-effects Reported:
2	Bendroflumethiazide induced diabetes (told by diabetic nurse she should not have been taking it as it can induce diabetes if you're already at high risk). Paracetamol causes itching.
23	Tablets caused constipation, went back to the GP who gave senna.
32	Simvastatin stopped was causing cramps, headache, insomnia and back pain.
40	Bloated.
41	Diagnosed with high blood pressure after taking naproxen long term.
54	Weight gain caused by medication.
71	*"If I get any side-effects from doctors' medicines I just do not take them for a while, Son is a Doctor so makes sure its ok to stop medicines."*
85	Side effects with tablets doctor gives (metformin for diabetes), so has had doses reduced from one to half a tablet twice daily. Did try and stop taking the tablets but nurse said tablets must be taken for diabetes.
87	*"Supposed to be on fifteen tablets daily, for high blood pressure and diabetes but so many side-effects I have stopped them all and feel much better. When you take Western medicines expect more side-effects and tablets to follow."*
100	Nausea from taking contraceptive pill.
111	Got diarrhoea from her medicines, went back to the GP who changed the tablet for an alternative.
124	Bad reaction to diclofenac, had to go back to see the doctor.
130	Palpitations caused by medicines.
144	Unpleasant stomach ache from the contraceptive pill so went back to GP who gave an alternative pill, still has the stomach ache but not been back again.
153	Hair was falling out with salbutamol inhalers so went to GP who gave a different inhaler.

CWM have a substantial amount of clinical data and evidence to support their use. All the side-effects experienced during and after clinical trials are reported (pharmacovigilance); consequently warnings are issued in a patient information leaflet, so patients are aware of side-effects associated with their medication. On the other hand, it could be argued that because HMs do not have the same level of clinical evidence to support their use, their side-effects are not recorded and therefore not as well known. This research has documented some of the side-effects associated with the consumption of HMs, and defined concepts such as '*garam*' and '*bhye*' which may help people recognise and identify adverse effects from HMs.

In summary of this section about the use of HMs alongside CWM, it can be noted that people will try a variety of remedies (traditional, conventional, or both) to supplement, maintain, and treat their health. There are barriers which prevent people from talking to HCPs about their use of HMs; for example, language and cultural differences which make explaining traditional concepts to Western HCPs difficult. Such issues need to be addressed to improve relationships between the ethnically diverse population and HCPs. The lack of HCPs' knowledge and understanding of HMs could be tackled by providing more training and raising awareness of the issues identified (Newall et al., 1996). There is a clear difference in the incidence of side-effects between HMs and CWM; whereby HMs have fewer and less serious side-effects in comparison to CWM. The results from this analysis have given an insight into some of the potential adverse effects and risks of taking HMs and CWM together. Valuable research like this could help raise awareness of the issues identified and promote the safe and effective use of both HMs and CWM.

The following section contains results from all 192 participants surveyed.

2.6.4 Treatment of Minor Health Problems

Minor ailments are considered to be conditions such as allergies, coughs, colds, backache, toothache, headaches and migraines, indigestion, and skin problems; in most cases people manage these conditions through self-care using over-the-counter (OTC) products (PAGB, 2009). The treatment of minor ailments is costing the NHS £2 billion per year (PAGB, 2009); hence, Government initiatives to promote self-care for minor health problems are encouraging people to manage their own health (Local Government Association, 2013). The use of HMs is becoming more popular for minor and even more serious health problems, but the extent of which HMs are used to treat minor ailments remains unknown (Newall et al., 1996; Barnes, 2003). The final section of the questionnaire was designed to gain insight into how SA populations in the UK are managing their minor ailments, and to see where participants go for advice. Moreover, it was an opportunity to explore participants overall opinions on the efficacy of HMs in comparison to CWM.

Question 13: ***Do you use herbal remedies for minor health problems before seeking medical help?***

Participants were asked if they used herbal remedies before seeking medical help for minor health problems such as a cold or headache. A substantially large proportion of the research population (72%, n=139) claimed they did, while just 28% (n=52) said they did not. The first question of the survey began by asking whether or not any homemade herbal remedies were used to supplement participants' health, to which 66% (n=126) of participants said '*yes*'. Question 13 is structured slightly differently but shows more participants claimed to use herbal remedies by the end of the survey. This could be because as the questionnaire progressed it reminded participants of HMs they were familiar with. Consequently, when asked the question of whether or not they used herbal remedies for minor health problems more people said yes by the end of the survey.

Participants were then asked what they would do in the event of a minor illness and who they would seek advice from. A ranking order style of question was used for question 14 and 15 to determine in which order participants would take what action. This was to allow participants to decide which action was most appropriate for them.

Question 14: ***In which order would you consider the following treatments for a minor illness:***

Participants were asked what they would do for a minor illness such as a sore throat or headache, they were given four options: not consider taking any treatment, self-medication using herbal products, self-medication using over the counter (OTC) products from the pharmacy, or consult their doctor for advice and medication. They were then asked to rank the options one to four in the order they would consider the actions (i.e. one being the first option and four the last). Figure 2-25 demonstrates the ranking of each of the options by the different age groups. The first choice for 44% (n=84) of participants was to self-medicate using HMs; this was the most popular first choice for many of the elderly respondents (n=37) who may have more experience and trust in HMs. Similar work conducted by Bhopal (1986a) found participants were more likely to take 'Asian' herbal remedies than CWM for conditions such as abdominal discomfort, diarrhoea, toothache and earache. Likewise, research by Sandhu and Heinrich (2005) which looked at the use of Sikh HMs uncovered 38% (n=32) of their participants said the first option for treating a minor illness for was by self-medicating with HMs. Other options considered first for minor ailments in this research included: not taking any treatment by 39% (n=75) of participants, followed by 8% (n=15) who would go to the pharmacy for an OTC product, and 9% (n=17) who would see their doctor.

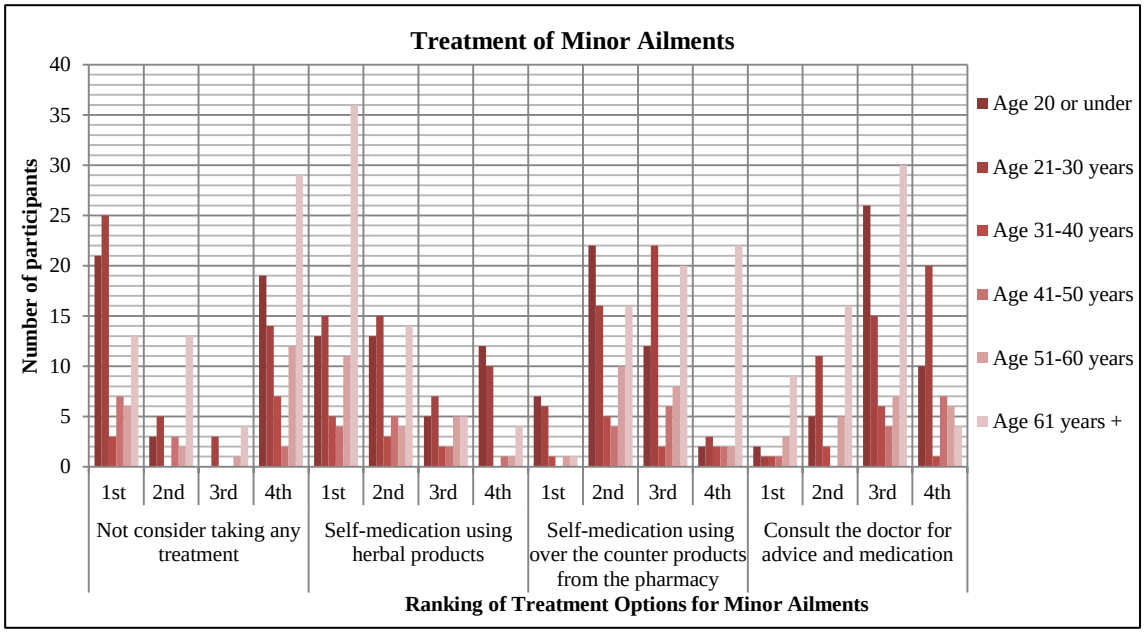


Figure 2-25 The Ranking Order of Treatment Options for Minor Ailments Categorised by Age Groups

Participants who said they would not take any treatment for a minor illness (as the first option) commented on the nature of the condition; stating that if it was a minor ailment it would resolve itself without the need for treatment. The Mintel Report (2009) also found that

'*a do nothing*' approach was prominent in the UK, where "*six in ten adults would wait for the ailment to run its course*" rather than taking any treatment or seeking advice. It should also be noted that the elderly research population in this study were the least likely to leave a minor illness without being treated. Often the older participants were taking several prescription medicines for various long term health problems so preferred to seek medical help from their doctor, rather than do nothing for a minor ailment; as delaying treatment could have serious consequences.

The younger age group of participants (under 20-30 years) were more likely to treat a minor illness with an OTC medicine from the pharmacy as their 2nd or 3rd option, than the other age groups. In general, to summarise the ranking order of the four possibilities: the first option for most participants was to self-medicate using herbal products (44%, n=84), the second selection was to self-medicate using OTC products from the pharmacy (38%, n=73), the third choice was to consult the doctor for advice and medication (46%, n=88), and the final option was to not consider taking any treatment (43%, n=83).

Question 15: ***Who are you most likely to seek advice from for minor health problems?***

The vast majority of participants (82%, n=157) said they would turn to their family and friends as the first port of call for advice for minor ailments (Figure 2-26); a pharmacist was the second option for 43% (n=83) of participants, followed by seeing a doctor as the third option (46%, n=88) and the last option for most participants was consulting a herbal practitioner (83%, n=160).

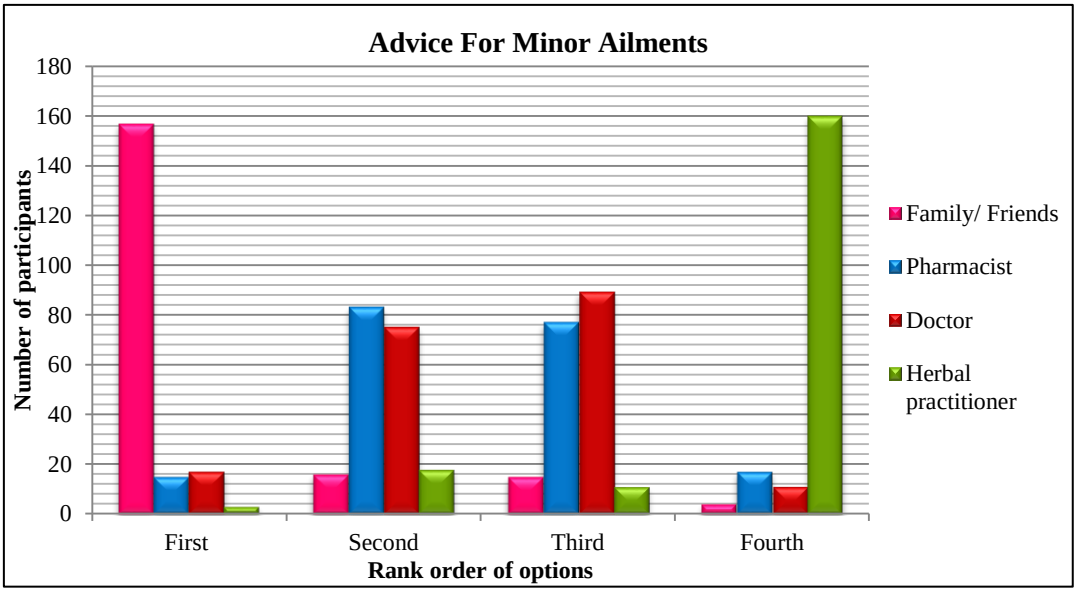


Figure 2-26 Ranking Order of Who Participants Would Get Advice for Minor Ailments From

The General Pharmaceutical council (GPhC) claims that pharmacies are a key source of information and advice for people which can be easily accessed (GPhC, 2015). Community pharmacists should be the first port of call for minor ailments, before seeking advice from doctors (Local Government Association, 2013). Participants in this study who said they would seek the advice from their pharmacist (8%, n=15) before the other options acknowledged the pharmacies were easy to access, and a product could be quickly bought without delay. However, the results of this investigation suggest a slightly higher proportion of participants would rather see their GP (9%, n=17) first than a pharmacist for advice for a minor illness. This was also demonstrated in work conducted by Hammond et al. (2004) who found that despite pharmacists having an increasing role in managing minor ailments people continued to see their GP. Similarly the Mintel report (2009) claims the doctor remains the first port of call for seven out of ten adults seeking medical advice. Sandhu and Heinrich (2005), found the majority of participants (58%, n=49) in their research said they would seek medical advice from their HCPs, either their own GP (29%), a family friend who was a GP (9%) or pharmacist (20%) as the first option for minor ailments. A survey conducted on behalf of the GPhC found that nine in ten people said they trusted the health advice from their pharmacist, although there was greater trust for their doctors; of those who preferred to seek advice from their GP many claimed it was a habit and that they did not even think to go the pharmacy (GPhC, 2015). Some of the reasons why people choose to see their GP over pharmacists were identified by research conducted by the PAGB (2009), including: the cost of buying OTC medication in comparison to a free prescription for those who are eligible, people feeling extra cautious when treating children, patients seeking reassurance from their GP, and people lacking knowledge about the pharmacists roles. Participants in this research who took regular prescribed medication (n=12) said they preferred to see their GP as their first option, because the doctor was aware of their current health condition so could provide better advice than a pharmacist who does not know them; in addition, they did not want to risk taking HMs in case of any adverse effects or interactions.

With the increasing popularity of HMs (Newall et al., 1996; Mintel, 2009), it would suggest there would be more demand for trained herbal practitioners (Chevallier, 2007). However, the sample surveyed was not interested in seeking the advice of herbal practitioners for minor health problems. This may be due to a lack of awareness of how to access traditional herbal practitioners in the UK, or the cost associated; as conveyed by participant 82 who said, *''I cannot afford herbal medicines they are too expensive to see a practitioner, Western medicine is free and easy to access here.''* This view was shared by several other participants too. The cost of HMs could be a key factor in determining participants' choice of treatment, which may also contribute to the changing attitudes and use of HMs and CWM.

Before the National Health Service (NHS) was established in 1948, it was very expensive to see a doctor and buy the prescribed medicines. The NHS introduced free healthcare to everyone, and this saw a shift from seeing the pharmacist for medicines for minor health problems to going to see the doctor (Royal Pharmaceutical Society, 2011). If people are entitled to free NHS treatment and medicines it appears they would opt for this instead of having to pay for alternative treatments; as Participant 126 stated, *"Why do you want to use herbal medicines when the doctor looks after you and you get everything free here?"* Johnson et al. (1983) and Bhopal (1986a) identified that SA communities would rather see Western HCPs than a traditional practitioner in the UK; this has also been highlighted from the results of this investigation.

Question 16: ***Would you prefer to use traditional herbal remedies over conventional Western medicines for minor health problems?***

This question was designed to explore participants' perceptions of what they preferred to take for minor health problems; 79% of participants (n=152) said they would prefer to use HMs for minor ailments. Pieroni et al. (2010) also found the majority of their Bangladeshi participants claimed they would prefer to use traditional medicines over Western medicines.

In this study there were some comments which suggested participants were willing to try alternative treatments but had reservations because of their lack of knowledge, and evidence based research to prove their safety and efficacy. As highlighted by participants 114 and 179 who said*, "I would prefer to use more herbal remedies if I had the knowledge"* and participant 132 who claimed, *''if there was more evidence on the safety of herbal remedies I would prefer to use them over Western medicines.''* Two young female participants mentioned that their parents (who were all born in the UK) only used CWM which meant they did not use or have any knowledge of HMs; they went on to say they would prefer to use HMs if they had better knowledge. Some participants remained uninterested in trying HMs, as exemplified by participant 188 who declared, *''there is not much evidence on effectiveness of herbal medicines, it might just be a placebo effect.''* This view was also shared by participant 191 who revealed he would rather take a prescription medicine than use any HMs. Several participants commented on the duration of action of HMs in comparison to CWM; conveying they would prefer to use HMs if they were as efficient as CWM, as mentioned by participant 143 who said, *''I would prefer to use herbal remedies for minor health problems if they worked as fast as Western medicines.''*

In discussions amongst participants, while the surveys were being conducted, there was some debate about how much evidence has guided the use of HMs, which have been used for longer than CWM. Participants spoke about their grandparents and parents giving them HMs from a young age, which worked, but there was no clinical evidence to support their use. These findings suggests evidence based medicine has become very important in guiding peoples decisions; similar conclusions have been proposed by many researchers including Evans (2008) and Pole (2008). The range of resources available to help people make an informed decision about their healthcare has become easier to access, and these have reinforced the ideas of using medicines which have been clinically approved.

Question 17 & 18: ***Which do you think are more effective for treating minor*** **(Question 18)** ***serious/ long term health conditions (Herbal or Western Medicines)?***

The final two questions aimed to get an insight into which system of medicine participants believed was more 'effective' for treating minor and serious health conditions. Minor ailments included short term health problems such as a cough, cold, headache or indigestion; whereas, serious illnesses included chronic conditions like hypertension or diabetes etc. Figure 2-27 shows a clear distinction between the perceived effectiveness of HMs and CWM for minor or serious conditions. Most participants (65%, n=125) believed HMs were more effective than CWM for minor health problems. Comments about HMs being natural and therefore safer with less side-effects were noted; as well as the long standing use of herbal remedies amongst participants' families. Although, some participants had their reservations and expressed their concerns of the insufficient evidence to support the use of HMs; for example, Participant 160 claimed, *''the effectiveness of herbal remedies can only be determined if they are well established.''* While others mentioned their lack of knowledge and experience with using HMs so felt they could not comment on the efficacy of HMS, as they had only ever used CWM.

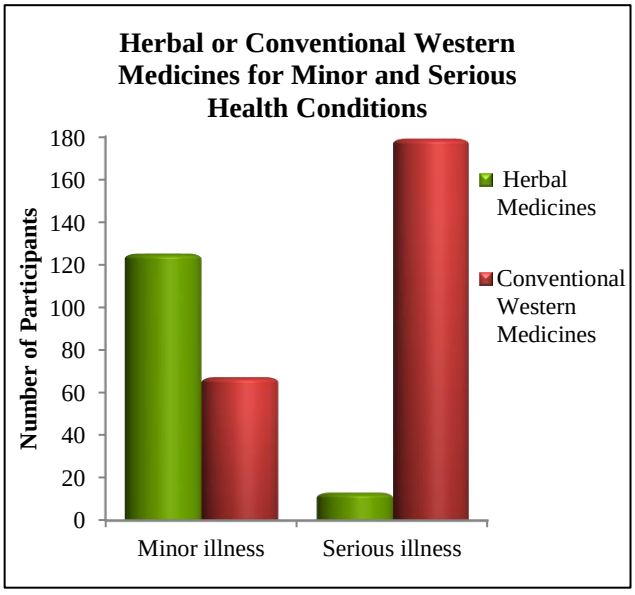


Figure 2-27 Participants' Views on the Efficacy of Herbal and Conventional Medicines for Minor and Serious Health Conditions

When participants were asked which type of medicines they thought were more effective for more serious health problems, 93% (n=179) of participants were in agreement that CWM were more effective than HMs. This is supported by findings by Bhopal (1986a) who discovered Asian communities preferred to use Western medicines over traditional herbal remedies; and Johnson et al. (1983) who found that Asians in the West Midlands did not perceive traditional HMs to be superior to CWM, in fact they felt conventional medicines were better. Participants in this research who suffered from long term serious health problems such as diabetes and cardiovascular disorders made reference to feeling obliged to take their prescription medicines; due to fears of what their doctor would say if they did not comply and the health consequences of not taking their medicines. Others expressed their trust in CWM due to the rigorous clinical testing and evidence to prove their safety and efficacy.

Despite the mass consensus, a small fraction of participants (7%, n=13) believed that HMs were more effective than CWM even for long term serious health problems. This included participant 122 with end stage liver cancer who said her chemotherapy treatment was making her worse and it was the HMs she was taking that were keeping her strong. Wachtel-Galor and Benzie (2011) found the use of HMs increased in patients with terminal illnesses such as cancer. Some participants in this research sample who were in favour of HMs for minor and serious conditions made references to how valuable HMs were and noted that some CWM were based on traditional HMs. Equipped with the right knowledge, these participants believed they could manage their own health without the need for CWM; they believed that natural remedies were intended to be used to treat illnesses and were safer with fewer adverse effects. Similar results have been observed by other researcher who have looked into the perceived safety and efficacy of HMs and also found that people think HMs are natural and so safer (Ipsos MORI, 2008; Mintel, 2009; Jennings, 2014).

There is a clear division in opinions as to which form of treatment is more effective for minor and serious health problems. Most participants felt HMs were effective for minor ailments while CWM were more effective for serious conditions. The results highlight that the research participants had a great wealth of knowledge of HMs which has been passed on through the generations. The use of HMs amongst SA communities in the UK remains prominent and it can be concluded that participants use both HMs and CWM to treat, manage, and maintain their health.

2.7 South Asian Traditional Medicines Project Summary

This research set out to explore the origins, use and transmission of knowledge of HMs amongst SA diasporic communities in the UK. Two hundred surveys were conducted across several locations in Birmingham and Leicester of which 96% (n=192) were used in this analysis. Although the research was conducted in two cities participants from across the UK took part in this questionnaire; hence, the results can be generalised to SAs in the UK. The research sample was composed of people from different age groups, ethnicities, and religions. The methods used to conduct this research enabled the aims and objectives to be successfully achieved. As the questionnaires were all administered by the primary researcher the importance of remaining impartial and not influencing participants responses was imperative; the skills obtained while doing this have been highly valuable.

One of the key findings of this investigation was that 66% (n=126) of participants claimed to use HMs to supplement their health; this increased to 72% (n=139) of participants who said they used HMs for minor ailments, by the end of the survey. Participants from all of the different age, religious, and ethnic groups claimed to use HMs. Unlike the Ipsos MORI report (2008) which claimed the use of HMs was more popular with people educated to degree level or above, the use of HMs amongst this research population was not affected by their academic status; participants with no qualifications and those with degrees or above all claimed to use HMs. A difference in the use of HMs for minor ailments was seen between male and female participants; whereby, more female participants (78%, n=103) than male (60%, n=36) said they used HMs for minor ailments. This is supported by the results of the Ipsos MORI report (2008) which found 41% of female compared to 29% male participants claimed to have used HMs. The Mintel report (2009) also highlights that women are more likely to use HMs than men; in addition, Ernst (2000) claims women generally use HMs more than men. When participants were asked if they preferred to use HMs or CWM for minor ailments the vast majority of participants (79%, n=152) said they would prefer to use HMs; several participants did comment on their lack of knowledge of HMs which prevented them from using HMs. HMs were considered to be more effective than CWM for minor ailments by 65% of participants (n=125); whereas, for more serious health problems CWM were deemed more effective by 93% of participants (n=179). Just 7% of participants (n=13) believed HMs were more effective than CWM for serious illnesses. These results signify that both HMs and CWM have an important role in the management of participants' health.

The variety of HMs participants used to prevent, treat and maintain their health included: ginger for nausea and colds, fennel for digestion, lemon to lighten the skin and remove scars, karela for diabetes, and cinnamon to reduce high cholesterol. The use of fresh or dried fruits,

vegetables and herbs, oils, teas, seeds, syrups, creams, and gels were recorded by participants. The most popular item recalled by 109 participants (57%) was turmeric; its immune boosting properties, antibacterial effects for wound healing, and anti-inflammatory action in arthritis were frequently documented by participants. Several formulations of how to take turmeric were noted, the most popular method (cited 45 times) for consuming turmeric orally was to mix a spoon of turmeric in warm milk (with the optional addition of butter or oil, and black pepper). Even though turmeric has been used to treat numerous health conditions long before written records were created, some of the health benefits of turmeric are now being extensively investigated by researchers (Ravindran, et al., 2009; Sikora et al., 2010). Some of the valuable knowledge of traditional HMs from participants has been documented and summarised in Appendix 5.

Participants' knowledge of HMs was assessed by calculating the number of different herbal remedies, products or ingredients they recalled throughout the questionnaire. Just 5% (n=10) of participants were unable to list any herbal products; the average number of products recalled per person was ten, while the highest number of items recalled by one participant was thirty six. This data highlighted participants wealth of knowledge of HMs. Results of the independent sample t-test revealed there was a significant difference ($p=0.001$, 2-tailed) in the knowledge of HMs between male and female participants; the mean (M) number of responses per female participant was 11 ($SD = 7.01$), while for males $M= 7$ ($SD = 6.18$). Throughout history, across different cultures around the world, women have been given the responsibility of the families' health and prosperity (Ehrenreich and English, 1973; Chaudhury and Rafei, 2001; Hegg, 2013); this could be one of the reasons why women have more knowledge of HMs than men. The Mintel report (2009) also claims that females have more knowledge of HMs in comparison to males. A comparison of age and knowledge of HMs was conducted using an ANOVA test (one-way between-groups); it uncovered a statistically significant ($p = 0.0003$) difference in knowledge between the different age groups, suggesting older participants had more knowledge of HMs than younger participants. Despite this participants from all age groups did have an awareness of the potential uses of many different HMs.

The availability of SA ingredients to formulate HMs and commercial HMs is increasing in the UK, making it easier to obtain and use traditional remedies; as portrayed by participants in this study. Asian and Western chain supermarkets, health stores, and online retailers are trying to cater for the needs of migrant communities, in the UK, by stocking ethnic ingredients (Retail Think Tank, 2013). However, some herbal products were identified as being difficult to source in the UK; therefore, participants claimed they had to obtain their ingredients from other places such as the internet or abroad. An example of a plant which

was difficult to cultivate in the UK was Tulsi (*O. tenuiflorum* L.). Participants mentioned how dried Tulsi leaves were imported to the UK from India and Africa; or culinary basil (*O. basilicum* L.) was used as a substitute as this was the closest alternative participants could find in the UK. The import and use of HMs from foreign countries has its own risks, such as accidental or deliberate species substitution or adulteration (Pharmaceutical Press, 2013). As identified in the studies by Seethapathy et al. (2015) and Kumar et al. (2015) who identified that the Indian herbal medicines market is affected by species substitution and adulteration. The applications of DNA barcoding and authentication of herbal medicines in the UK is limited, this research will contribute some important information to this discipline.

The significant majority of participants (85%, n=163) said that HMs were part of their traditional family and cultural heritage. It was uncovered that the family remains the primary source for transmitting traditional knowledge of HMs, as 80% (n=101) of participants claimed to have learnt about HMs from their family. In addition, 82% (n=157) of participants said they would seek advice from their family first for minor ailments; this signifies the importance of the family support network which has a vital role in transmitting traditional knowledge of HMs (Chhetri, 1994). Bhatia et al. (2014) recognises that knowledge of HMs has developed through age old experience and has been orally transmitted from one generation to another; hence, if the knowledge of HMs is not communicated with younger generations or documented, there is a risk of it being forgotten. This research has documented some of this important traditional knowledge. Despite the family being the most common way of learning about HMs, some participants (20%, n=25) claimed to have learnt about HMs from their religion, school, friends, the television, radio, internet and social media (Facebook, Twitter and YouTube); these results identified diverse ways of learning about HMs.

When participants were asked if they took any regular prescribed medication, 55% of participants (n=106) claimed to take prescription medication for a variety of health conditions such as: asthma, arthritis, cardiovascular disease, diabetes, and thyroid disorders. Participants who consumed HMs alongside their CWM were then asked if they shared this information with their HCPs; 69% (n=73) revealed they did not tell their doctor, while 82% (n=87) said they did not tell their pharmacist. Some participants feared they would be treated differently or not at all if their HCPs knew they were taking HMs at the same time as CWM. A few participants commented on language and cultural barriers which prevented them from sharing information about their use of HMs with their HCPs; concepts such as *garam* and *bhye* which do not exist in Western medicine were difficult to explain to HCPs. Heuschkel et al. (2002) revealed that just 24% of patients in their survey initiated conversations with their doctors about HMs; similarly, Bhopal (1986a) found participants did not think it was

important to tell their HCPs about their use of HMs. During the analysis of the data, several potential interactions between HMs participants used and CWM participants were prescribed were identified. If participants do not tell their HCPs about their use of HMs it could affect their pharmaceutical care (Pharmaceutical Press, 2013). A crucial discovery of this research is that a large number of SA participants do not tell their HCPs about their use of HMs; reasons why they did not share this information were also documented. HCPs perspectives will be considered in the following chapter (Chapter 3).

The wide spread use of HMs in developing Asian countries where herbals may be more affordable in comparison to conventional medicines is well known (WHO, 2008); however, in the UK where conventional medicines are often free (i.e. for patients over 60 years old, or people with certain conditions such as diabetes or epilepsy) (PSNC, 2015) HMs may actually be a more expensive alternative. This was identified by some participants in this research who were not interested in using HMs as they received free NHS treatment. It was clear that some participants in this study consumed HMs alongside their prescribed CWM; 78% (n=83) of participants who took prescribed medicines claimed to use HMs intermittently or regularly to supplement their health, treat an ailment, or to counteract side-effects from CWM. When considering the side-effect profiles of CWM and HMs, significantly more participants said they experienced side-effects from CWM (43%, n=46) than from HMs (13%, n=16). Participants commented on HMs being natural, safer and with fewer side-effects, this view has been documented in many other studies too (Ipsos MORI, 2008; Mintel, 2009; Ravindran et al., 2009). Pole (2008) argues that herbals are not safe because they are natural; they are safe because in Ayurveda after the correct diagnosis, the right herbs can be prescribed. This reiterates the importance of having the knowledge to be able to use HMs safely, which some participants in this study felt they lacked. Participants believed if more literature and evidence based research was available to help educate them about HMs it might encourage them to try alternative remedies.

Traditional knowledge of HMs has been created via trial and error and has been perfected over time, unlike conventional medicines which have been studiously researched. One issue identified by participants in this study was that the safety and efficacy of HMs has rarely been demonstrated using modern scientific investigations. An evidence-based approach to this issue needs to be employed in order to provide validation for remedies which have been used throughout history (Pole, 2008). Similarly, Evans (2008) explores how evidence based medicine has become influential in decision making; modernisation of HMs could be the way to integrate the two individual systems. Xie (2001) claims traditional medicine contains some valuable elements but there are parts which are no longer applicable and so suggests the need for scientific research to decide which elements to keep.

Similar research to this investigation was conducted by Bhopal (1986a) who explored the relationship between traditional and Western medicine in an Asian community in Scotland. This research was on a much smaller scale, it was limited to an Asian community in Glasgow, and is now very dated. Since then there have not been many other ethnopharmacological surveys to explore the current use of HMs in SA communities, in the UK (Pieroni et al, 2007; Pieroni et al, 2010). The results from this questionnaire have provided a detailed insight into the current use of HMs amongst SA diasporic communities, in the UK. The use of traditional herbal remedies and ethnobotanical research play an important role in scientific developments (Malla and Chhetri, 2009); therefore, it is imperative to record this knowledge. It has been suggested that ethnic people have immense knowledge of HMs which they inherit and pass on from generation to generation, through oral conservations; however, there is a risk of losing this knowledge if not documented (Chhetri, 1994; Thring and Weitz, 2006; Bhatia et al., 2014). The results of this questionnaire have enabled some of this precious information to be recorded.

The analysis of the results has revealed several areas which require further investigation; for instance, the identification and authentication of herbal products to ensure the correct species are used in HMs, and HCPs' views and opinions of HMs. These concepts will be explored further in the following chapters.

3.0 Chapter 3 – Healthcare Professionals' Views of Traditional Herbal Medicines

3.1 Introduction

The South Asian Traditional Medicines (SATMED) questionnaire (Chapter 2) revealed some interesting findings about South Asian (SA) people in the United Kingdom (UK) taking traditional herbal medicines (THM) and whether or not they shared this information with their medical doctor or pharmacist, i.e. their healthcare professionals (HCPs). A key discovery was that the vast majority of participants did not tell their HCPs of herbal medicines (HMs) they consumed at the same time as taking prescription medicines; 69% (n=73) of participants who were taking conventional Western medicines (CWM) as well as HMs said they did not tell their doctor, while 82% (n=87) did not tell their pharmacist. Various reasons for not telling their HCPs about the concurrent use of HMs and CWM were uncovered, such as: a fear of being treated differently, not being treated at all, and being labelled as 'backwards' or uneducated. The SATMED questionnaire was able to extract some valuable information about the current use of HMs amongst diasporic SA communities in the UK; and to establish participants' views of safety, efficacy, and side-effects caused by HMs and CWM.

Further to this investigation, the primary researcher was intrigued at looking into what HCPs thought of THM and their experiences with HMs. By asking HCPs to share their opinions on the safety and efficacy of HMs, and their knowledge of interactions and adverse incidents from combining HMs with CWM, both patients and practitioners perspectives could be assessed. It was anticipated that this study would reveal information about HCPs personal and professional views of HMs, something which has not been greatly explored in the UK before (Freymann et al., 2006). There is extensive research available on HCPs in the United States (US) and other European countries but limited information from the UK (Berman et al., 1998; Smith, 2011). Hence, this research will help to contribute to the lack of information on UK based HCPs use and opinions of HMs.

The use of social media in communicating, networking, and even health promotion is expanding (Thackeray et al., 2012). Mediums such as Facebook and Twitter were considered as an innovative way to raise awareness of this research and to distribute the questionnaire. The use of social media in conducting social research is being recognised as a valuable tool

for data collection (Wilson et al., 2012), and was used to get HCPs across the UK involved in this study.

3.2 Aim of the Healthcare Professionals Survey

The aim of this investigation is to explore healthcare professionals' personal and professional opinions and experiences of traditional herbal medicines.

3.3 Objectives of the Healthcare Professionals Survey

1. Explore healthcare professionals' personal use and knowledge of traditional herbal medicines.
2. Investigate healthcare professionals' professional experiences of patients taking herbal medicines.
3. Look into the impact of combining traditional and Western medical systems.
4. Uncover healthcare professionals' professional opinions on the safety and efficacy of herbal medicines.

3.4 Background to the Research

By reviewing the current literature available around this topic it will help to explain the importance of this study (i.e. supporting the need to conduct and document this research). A definition of what a healthcare professional (HCP) is will describe the criteria for the research population. Exploring the current education standards for UK HCPs to study complementary and alternative medicines (CAM) will give an insight into how HCPs are prepared to manage patients using alternative therapies. The relationship between HCPs and their patients and the implications of combining HMs and CWM will be considered. The use of social media to conduct scientific research will also be examined.

The terms THM and HMs will be used interchangeably.

3.4.1 Definition of a Healthcare Professional

The International Labour Office (ILO) created a standard definition of occupations called the International Standard Classification of Occupations (ISCO). The latest version was revised in 2008 (ISCO-08). The objectives of the ISCO-08 were to standardise and organise

occupations so they are universally recognised. The ISCO-08 definition for health professionals is: *"Health professionals study, advise on or provide preventive, curative, rehabilitative and promotional health services based on an extensive body of theoretical and factual knowledge in diagnosis and treatment of disease and other health problems. They may conduct research on human disorders and illnesses and ways of treating them, and supervise other workers''* (International Labour Office, 2012; WHO, 2014). The list includes: medical doctors (from all disciplines including general practitioners to specialist physicians), nurses, midwives, paramedics, dentists, pharmacists, environmental and health professionals, physiotherapists, dieticians, nutritionists, audiologists, speech therapists, optometrists, podiatrists, occupational therapists, and traditional and complementary medicine professionals.

In the UK the National Health Service Reform and Health Care Professionals Act 2002 has outlined that HCPs are those regulated by bodies such as the General Medical Council, General Dental Council, General Optical Council, General Osteopathic Council, General Chiropractic Council, General Pharmaceutical Council, and the Nursing and Midwifery Council (National Health Service, 2002). In August 2012 The Health and Care Professions Council (HCPC) was created to regulate several professions, including: arts therapists, biomedical scientists, chiropodists/ podiatrists, clinical scientists, dieticians, hearing aid dispensers, occupational therapists, operating department practitioners, orthoptists, paramedics, physiotherapists, practitioner psychologists, prosthetists and orthotists, radiographers, social workers in England, and speech and language therapists (Health and Care Professions Council, n.d.).

According to UK standards HCPs will be regulated by a professional body; however, the ISCO-08 does not specify such details, it just standardises what occupations are classed as being HCPs. From this is can be concluded that the term 'healthcare professional' is a very broad term and refers to someone who is involved in diagnosing, treating, caring or rehabilitating, and reviewing patients health. HCPs have a vital role in health promotion and a duty of care to their patients, meaning they must be able to advise patients about various issues appropriately. As the popularity of HMs increases Western HCPs must have some insight into HMs to ensure patients are cared for properly (Zhang et al., 2012).

3.4.2 Academic Training in Complementary and Alternative Medicines

Complementary and Alternative Medicines (CAM) is a very broad term which covers over 700 types of therapies (Kayne, 2002) including: diet and exercise, reflexology, massage, relaxation techniques, and HMs. As CAM is becoming more popular in the UK, and more people are experimenting with holistic treatments they are becoming more professionalized (Clarke et al., 2014). The House of Lords report (2000) highlighted that conventional HCPs should be familiar with CAM therapies, including the potential uses and risks. Some CAM therapies are already accepted and widely incorporated as part of conventional Western medicine systems such as acupuncture, chiropractic and osteopathy; these are well regulated by the Acts of Parliament and are now included in the NHS and NICE guidelines (Clarke et al., 2004; Cant et al., 2012). Other forms of CAM such as HMs are becoming a focus for regulation; but, the extent of which this knowledge of CAM is taught to undergraduate HCPs remains to be discovered. While searching the literature for information about the education of HMs content in medical and pharmacy courses in the UK, it proved difficult to assess how much information was transmitted to undergraduate students about HMs. Subject guides, course and module information from numerous schools were examined with no possibility of determining what future HCPs were taught about HMs. In addition, any research on undergraduate courses in the UK looked at CAM as a whole and did not distinguish between the different elements such as physical or biological therapies.

Regulatory bodies such as the General Medical Council (GMC) which regulates doctors, General Dental Council (GDC) which regulates dentists, the Nursing and Midwifery Council (NMC) which regulates nurses and midwives, and the General Pharmaceutical Council (GPhC) which regulates pharmacists all have a role in setting standards for education of undergraduate students (i.e. the future HCPs). The GMC (2009) updated its guidelines for undergraduate medical education in *Tomorrow's Doctors* for application from 2011-2012, these are the current guidelines in place. One of the objectives under 'prescribe drugs safely, effectively and economically' states courses must, "*Demonstrate awareness that many patients use complementary and alternative therapies, and awareness of the existence and range of these therapies, why patients use them, and how this might affect other types of treatment that patients are receiving*." The GPhC standards for the initial education and training of pharmacists called '*Future Pharmacists*' updated in May 2011, has a single statement *'complementary therapies'* in the indicative syllabus under section A1.1 *'How Medicines Work'*. The GMC, NMC, and GPhC have set very broad statements which institutes must include in their syllabi (GMC, 2009; NMC, 2010; GPhC, 2011); unlike the GDC which does not have any requirements for undergraduate dentistry students to learn about complementary therapies (GDC, 2015). Although some regulatory bodies do state

some CAM education needs to be included in the teaching syllabi the requirements are very broad and need to clarify the extent of coverage which is adequate for HCPs. The House of Lords Report (2000) claims the current level of CAM education is too uneven.

Many researchers around the world have tried to explore the extent of CAM education. In the US the majority of medical schools now offer courses on alternative medicine (Eisenberg et al., 1998). Wetzel et al. (1998) contacted 125 medical schools in the US to establish the provisions for CAM education, of the schools that replied, 64% (n=80) reported offering elective courses in CAM; although, the content and styles of teaching was variable. An online survey conducted by Ho et al. (2013) explored evidence based medicine (EBM) and CAM teaching in UK medical schools; 95 students replied representing 25 different medical schools, of which 54% said they had received lectures on CAM in their core courses. In Europe CAM education is part of the regular curriculum in 40% of medical schools and 72% of health science departments (Barberis et al., 2001; Vagra et al., 2006). Research by Smith (2011) investigated the views and practices of UK medical schools inclusion or exclusion of CAM in undergraduate medical courses; Smith (2011) found that all of the respondents to the survey (18 out of 31 medical schools in the UK) said CAM was included in their syllabus in some way. Institutes claimed the study of HMs was available as a special study module which students could select, while others claimed that CAM was formally taught. The extent of teaching ranged from a single lecture, student centred learning (coursework), presentations, and several mixed methods of coverage throughout the course. The amount of coverage a single lecture can divulge on CAM is questionable. The decision to include CAM in the academic syllabus may be influenced by several factors including: concerns of safety and efficacy, lack of research in the area, resources, and funding (Wetzel et al., 1998). The main reason for including CAM in undergraduate courses appears to be due to requirements by regulatory bodies such as the GMC and GPhC. However, there appears to be several reasons why this topic is not covered in great detail. Smith (2011) discloses various reasons for not focusing more on CAM in undergraduate medicine degrees in the UK, including: lack of interest by students, not enough students interested means running the course is not sustainable, lack of staff interest, no space in the syllabus or teaching programme as it is already stretched, and limited funding and resources to cover the content. Smith (2011) claims there was a consensus for staff to want to focus on evidence based medicine rather than CAM.

Pokladnikova and Lie (2008) looked at the attitudes of first and third year pharmacy students in the Czech Republic and found that 83% (n=231) were interested in learning about CAM and between 78-91% of students supported CAM education as part of their curriculum. Similarly in the UK Freymann et al. (2006) explored the knowledge and use of CAM in

undergraduate pharmacy students in The School of Pharmacy, University of London. They uncovered students were taught about CAM in the second year of the undergraduate course; however, it was still significantly less than the level of CAM education in other European countries. Students strongly agreed that HCPs should be aware of natural remedies. Inevitably, most HCPs will come into contact with patients who are interested in or using HMs; therefore, some provisions for educating future HCPs need to be in place (Pokladnikova and Lie, 2008; Smith, 2011; Ho et al., 2013).

3.4.3 The Relationship between Healthcare Professionals and their Patients

The SATMED questionnaire results revealed that participants taking HMs were not always willing to share this information with their HCPs; several reasons such as, a fear of being deemed backwards or treated differently were mentioned by participants. More importantly, factors such as language and cultural barriers were evidently affecting relationships between SA participants and their HCPs. Participants stated that their doctors did not understand them, and they found it difficult to communicate information about their health problems to Western HCPs as they were not familiar with the conditions. This was also uncovered by research by Eisenberg et al. (2001) who found participants did not disclose their use of HMs to practitioners because they felt like HCPs '*would not understand*'. This highlights the importance of having an awareness of cultural diversity; this is also part of GMC (2009) requirements in the *Tomorrow's Doctors* guide which states that, "*students should have acquired respect for patients and colleagues that encompasses, without prejudice, diversity of background and opportunity, language, culture and way of life."* Dogra et al. (2005) claim the importance of teaching cultural diversity needs to be developed further to ensure future HCPs can deal with the multicultural patient population.

The Ipsos MORI survey *'public perceptions of herbal medicines'* was carried out on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA) in 2008. The two strands of their investigation involved the qualitative exploration of people's views of HMs (the use, benefits, and risks) and a quantitative survey of the general public's use and attitudes towards HMs. The quantitative section involved face-to-face interviews with 2,032 adults in the UK; the results revealed that 35% of the survey population had used HMs. The majority of respondents were aware that there were risks associated with using HMs concurrently with CWM; thus, 56% of participant thought it was important to tell their doctor about HMs they were using, when they went to see their doctor. In addition, they found Asian and black and minority ethnic (BME) groups were the least likely to tell their

doctor about the use of HMs, and the most likely to believe it was ok to take the two forms of medicine together. Although pharmacists are 'drug specialists' and are in an ideal position to monitor and support the safe use of HMs it was clear people trusted doctors more in the Ipsos MORI survey, which concluded that participants trusted their doctors for advice on HMs more than pharmacists. The SATMED results also identified that more participants told their doctor (31%, n=19) rather than pharmacist (18%, n=5) about their use of HMs.

Negative attitudes of HCPs could affect the relationship with patients as they may not feel comfortable talking about their use of HMs (Robinson and McGrail 2004; Staines, 2011). This was echoed by participants of the SATMED questionnaire who felt that Western HCPs did not approve of their use of HMs. It is clear that patients may feel reluctant to talk to HCPs about their use of HMs if HCPs attitude towards HMs is disapproving. Awodele et al. (2012) revealed that in Nigeria 40.7% (n=122) of doctors would discourage their patients from taking HMs, various reasons such as: lack of traditional healer education and scientific research on HMs were noted as reasons for not promoting the use of HMs by HCPs in Nigeria. Robinson and McGrail (2004) found that patients did not disclose their use of CAM to HCPs as they were worried practitioners would be prejudice towards them and their negative responses would persuade them against using CAM.

A study into the use of CAM in children and young adults with inflammatory bowel disease (IBD) revealed that only 24% of patients using CAM had informed their doctor that they were doing so (Heuschkel et al., 2002). Studies into the use of CAM in children with cancer claim a substantial proportion of children use alternative therapies and the use of these therapies is often not discussed with their HCPs (Sawyer et al., 1994). Eisenberg et al. (2001) found that 60% (n=304) of participants claimed they did not tell the doctor about alternative therapies they used as the doctor never asked. Research conducted by the Drug and Therapeutics Bulletin (DTB, 2010) found that 77.3% (n=127) of HCPs surveyed claimed they were worried that patients would take HMs and not tell them (DTB, 2010). If patients do not want to talk to their HCPs about their use of HMs, should the responsibility of asking lie with the HCPs? Heuschkel et al. (2002) found that just 8% of doctors had initiated a discussion about CAM with their patients. Similar surveys conducted in Australia found that patients did not reveal their use of CAM as their practitioners never asked (Xue et al., 2007). The study by the DTB uncovered that only 12.9% (n=21) of HCPs in their survey said they '*always*' asked their patients if they took any HMs, 27% (n=44) said they asked '*on most occasions*' while just 8.6% (n=14) said they '*never*' asked about patients use of HMs (DTB, 2010).

People have been using HMs since before written records were created; the knowledge of medicinal plants has been passed on through generations and has had a key role in preventing and curing diseases. Inevitably, people cannot be told not to take HMs just because they lack clinical evidence, and people will not stop just because '*the doctor said so'*. With the migration of people from around the world, they have brought with them their own medicines and remedies to the UK, something which HCPs should be aware of and have some insight into (Dogra et al., 2005). There are risks and benefits of consuming HMs which HCPs need to recognise, especially if their patients are consuming products which they consider to be natural and therefore safe (Ipsos MORI, 2008). HCPs are at the forefront of primary care and need to be well equipped with the correct knowledge and training on HMs. It is not feasible for HCPs to advise patients about HMs if the background knowledge is lacking (Pokladnikova and Lie, 2008).

3.4.4 Taking Herbal Medicines alongside Western Medicines

There may be several reasons why people try or use HMs in addition to CWM, including: the traditional use of HMs passed through generations, dissatisfaction with CWM, a desire to take control of their own health by making their own decisions, and curiosity to try natural and holistic therapies (Doel and Segrott 2003; Clarke et al., 2004). Furthermore, people may use HMs when CWM do not appear to be working, or to treat and manage the side effects of CWM; this is commonly seen in cancer patients having chemotherapy and radiotherapy (Sawyer et al., 1994; Ernst and Cassileth, 1998). In developing countries the high cost of pharmaceutical medicines, hospitals and medical facilities means the use of HMs is popular, as they may be a cheaper alternative (WHO, 2000). However, in the UK where medicines and treatment is often free on the National Health Service (NHS) taking HMs may actually be more expensive. During the SATMED questionnaires distribution there were mixed views about taking HMs with regards to the financial cost; some participants said they were not interested in using HMs as they had to pay for them but got their prescription medicines for free. While others mentioned it was expensive to buy ingredients for HMs in the UK but it was cheaper to bring them back from countries like India and Pakistan. The import of HMs from foreign countries leads to further risks such as contamination, adulteration, and misidentification of species (Chapter 4).

HCPs views of combining THM with CWM are more likely to be determined by evidence based research and clinical guidelines, rather than the test of time (Heinrich, 2015). Awodele et al. (2012) disclosed that 41% (n = 123) of respondents in their survey to explore doctors view of THM, thought THM were effective for treating chronic conditions; although, none

of the doctors believed that THM alone could treat a patient. A comparison of UK and German general practitioners (GPs) attitudes towards CAM was done by Schmidt et al. (2002); they found GPs from both countries generally had a positive attitude towards CAM, although the Germans were more positive. GPs from the UK commonly referred patients for acupuncture, osteopathy and chiropractic treatment; while, significantly more German GPs referred their patients to herbal medicines. In the UK there is limited research to explore what HCPs think about combining HMs with CWM; this is an area which needs to be investigated further.

There is a growing concern of serious interactions occurring between HMs and prescribed or over-the-counter CWM (Vickers et al., 2006). Smallwood (2005) says there is a risk of people not taking their CWM correctly or as prescribed if they use HMs. Vickers et al. (2006) found participants did not think it was important to tell their doctors about their use of HMs as they did not think they interacted or affected one another. The Ipsos MORI report (2008) revealed that 66% of participants thought it was safe to take HMs at the same time as CWM. The notion that HMs are natural and therefore safe misleads people to think HMs are safe and free from deleterious effects (Howells, 1996; Staines, 2011). Eisenberg et al. (1998) claim 60-70% of CAM users do not discuss it with their doctors, meaning patients will not get the opportunity to learn about the potential risks and interactions of combining HMs and CWM (Schmidt et al., 2002; Graham et al., 2008). If HCPs are not aware that their patients are taking HMs alongside CWM they will not be able to intervene, or identify potential adverse effects and interactions (Heinrich, 2015). Furthermore, it may affect the appropriate pharmaceutical care plan from being implemented which may cause further complications to the patients' health (Ramsay et al., 2005).

3.4.4.1 Potential Herb-Drug Interactions

Polypharmacy is the term used to describe the simultaneous consumption of several medicines by one person (Duerden et al., 2013). Multiple medicines may need to be taken to control or treat health problems, in which case there is always the possibility for drug interactions to occur. The definition of a drug interaction is, "*when the effects of one drug are changed by the presence of another drug, herbal medicine, food, drink or by some environmental chemical agent,*" (Baxter, 2008). Dureden et al. (2013) highlights that polypharmacy can be harmful due to the increased risk of adverse drug reactions (ADRs), and impaired medication adherence if prescribed too many medicines. For CWM before any drug is licensed for use it undergoes rigorous testing, providing evidence for its clinical use. Details of potential drug interactions are also documented in several sources such as the

British National Formulary (BNF) and Stockley's Drug Interactions, which HCPs have access to. Various studies have found between 20-33% of the population use some form of CAM either alone or in addition to CWM (Freymann et al., 2006). Polypharmacy with CWM alone can be hazardous; but, by adding HMs it could further complicate the consequences. A herb-drug interaction is when the effect of a drug is altered by herbal products. Herb-drug interactions are based on the same principles of pharmacokinetics (i.e. affect the absorption, distribution, metabolism, or elimination) and pharmacodynamics (i.e. drugs effects are mimicked or antagonised) as with conventional drug interactions (Chavez et al., 2006).

Cytochrome (CYP) P450 are a group of enzymes which metabolise a variety of substrates and have an important role in drug metabolism. Enzyme-inducing herbals render drugs ineffective at sub-therapeutic levels as they are metabolised too fast. Alternatively when herbal products inhibit or reduce the rate of enzyme activity drugs will be metabolised slower than anticipated; this can cause the drug to accumulate and cause toxicity. Drug accumulation can be a serious problem especially for drugs with a narrow therapeutic range, such as digoxin (Colalto, 2010). One of the most well-known herbal products which affects the CYP 3A4 isoenzyme, is St John's Wort (SJW) (*Hypericum perforatum* L.); it acts as an enzyme inducer which reduces the plasma concentration of certain drugs including carbamazepine (anti-epileptic), ciclosporin (immunosuppressant), statins and oral contraceptives (Komoroski et al., 2005; Graham et al., 2008). Hyperforin one of the active constituents in SJW induces P-glycoproteins in the intestinal wall reduces drug absorption. Furthermore, if taken with Selective Serotonin Re-uptake Inhibitors (SSRIs) a type of antidepressant such as citalopram or sertraline, it can cause serotonin syndrome; where the levels of serotonin become so high in the body that it can have fatal consequences (Chavez et al., 2006; Williamson et al., 2013).

Fugh-Berman and Ernst, (2001) conducted a systematic review to explore the interactions between herbs and conventional drugs. They found that warfarin, a coumarin anti-coagulant, had the most interactions. Products such as garlic, gingko, ginseng, and even excessive consumption of green leafy vegetables can alter the anti-coagulant effects of warfarin (Chevallier, 2007). The mechanisms of how such interactions occur are not always understood or clinically significant; sometimes isolated reports of an interaction are the only indication of a potential interaction (Williamson et al., 2013). Warfarin is commonly initiated in patients who may have an increased risk of blood clotting, a history of a heart attack or stroke, or with prosthetic heart valves. It prevents the blood from clotting by inhibiting the conversion of hepatic vitamin K-dependent factors which reduce the amount of prothrombin produced; thus, reduces the rate of coagulation (Chavez et al., 2006). Foods which are high in vitamin K such as: cranberry juice, raisins, kiwi fruit, soya beans,

blackberries, blueberries, broccoli, and kale should be avoided by people taking warfarin (Fay, 2009). Despite being part of a healthy diet such products could affect the dose of warfarin required to have a therapeutic effect.

It is very difficult to establish interactions between HMs and CWM as they are not frequently reported (Chavez et al., 2006). This may be because people who use HMs and HCPs lack the knowledge of herb-drug interactions (Vickers et al., 2006; Staines, 2011). In addition, if HCPs are not aware of patients taking HMs they cannot intervene or identify potential interactions (Heinrich, 2015). Communication between patients and their HCPs about HMs is vital to be able to identify potential herb-drug interactions and adverse effects from HMs (Vickers et al., 2006). The incidence of interactions between HMs and CWM is not fully known, and currently there is no way of establishing potential problems or clinical outcomes (Williamson et al., 2013). The main way of monitoring the safety of HMs is via the MHRA, which set up the yellow card scheme (Barnes, 2003; MHRA, 2014a). Publications such as Stockley's Herbal Medicines Interactions (Williamson et al., 2013) and Phytopharmacy – An Evidence Based Guide To Herbal Medicinal Products (Edwards et al., 2015) could be essential in educating HCPs about potential herb-drug interactions; however, unlike the BNF and Stockley's Drug Interactions such texts are not readily available for HCPs to access.

3.4.5 Using Social Media to Conduct Scientific Research

The internet was made available to the public in 1991 with the introduction of the World Wide Web (WWW), which revolutionised how people learn, share information, and communicate (Vance et al., 2009). In the year 2000 just 26.8% of the UK population had access to the internet, in 2014 this had increased to 89.9% (*Internet Live Stats, n.d*). The launch of social media websites and applications such as Facebook, LinkedIn, Twitter, and YouTube have increased the ease and speed of communication; social media may be used for social, leisure, or professional communications. An Ofcom report in 2013 estimated that 82% of the UK population aged over 16 had access to the internet, of which 53% used social media websites (Ofcom, 2013). For researchers this presents exciting opportunities to conduct research as it can be used as a valuable tool to identify and recruit participants (Beninger et al., 2014); which can lead to a snowball sample for exploratory work (Bhutta, 2012). With the rise in popularity and access to social media, Murphy et al. (2013) claim that researchers should look into new ways to engage respondents and capitalise on this evolving phenomenon.

State public health departments have also adopted the use of social media to share information and health promotion updates with the public (Thakeray et al., 2012), they have used Facebook and Twitter as a way of communicating with the general public and YouTube channels to help promote health awareness. The NHS UK has over 87 thousand followers on Twitter, while the Department of Health UK have over 161 thousand followers (Twitter, 2015); the followers were comprised of a mixture of HCPs and the general public. A study by Valdez et al. (2014) explored the benefits and challenges of recruiting for research studies related to consumer health information technology using Facebook. The study was composed of two phases which aimed to recruit specific populations: the first targeted a particular ethnic group (Filipino-American) and the second focused on type two diabetics. They posted messages on target groups and pages, and also sent private messages to administrators and people within the groups to recruit them for their research. They found that using social media structures such as Facebook was good for small samples providing qualitative data; but not so good for large samples for quantitative research. On the other hand, Bhutta (2012) used her personal Facebook networks and obtained over 4000 completed surveys for her research within one month. Valdez et al. (2014) highlight the importance of creating trust and transparency to successfully recruit participants.

There are several advantages of conducting research using social media; it is a fast and efficient way of spreading a message, and getting instant feedback and replies (Sutton, 2010). Traditional methods of recruiting a sample can be time consuming and costly (Valdez et al., 2014). By using social media a large research sample can be recruited from different regions and even countries, allowing cross-cultural perspectives to be explored (Woodfield et al., 2013). It provides anonymity for participants who may not wish to disclose their personal details or identity, as they do not necessarily have to have any contact with the researchers (Beninger et al., 2014). In addition, as there is no facilitator present it eliminates the effects of researcher bias and may also give participants the freedom to express their views more open and honestly. It is cheaper than traditional research methods which may require printing and postage costs; in some cases it may even be free to set up an online survey. Once the research method, e.g. survey or focus group, is established it is easy to distribute the link to the survey and data can be collected quickly (Vance et al., 2009). There is an increasing number of people who have remote access to social media via multiple channels, e.g. smart mobile phones, netbooks, laptops, and tablets (Ofcom, 2013); therefore, online surveys can be completed quickly and instantly whilst on the go. Finally, as the data is collected electronically it reduces the risks of data input errors and is easier to analyse and store (Woodfield et al., 2013).

In spite of all the benefits of using social media to conduct research, there are also some disadvantages including: poor response rate, incomplete surveys, and the lack of control of the research population (i.e. cannot be sure of who is participating). This could be a particular problem if a specific research population is required but people who do not fall within the inclusion criteria participate. As the research is conducted without any face-to-face contact it may be difficult to keep participants engaged throughout the entire process. If participants do not understand a question there is no way of clarifying it, which may lead to unanswered questions or participants dropping out. The initial cost of some online research tools may be expensive, for example the basic survey on SurveyMonkey, an online survey development software, is free; however, this only enables a maximum of ten multiple choice questions and one hundred responses. In order to create a full survey where respondents can also write comments and unlimited responses can be collected it can cost from £229 per year (SurveyMonkey, 2014). Considering that just over 50% of British adults use social media it would mean a significant portion of potential participants are not included in the research (Woodfield et al., 2013). One of the main drawbacks of this type of research is that because it is a fairly new concept it is difficult to apply traditional ethical principles to (Beninger et al., 2014).

The Drug and Therapeutics Bulletin (DTB) conducted some research to explore what HCPs knew about HMs using online surveys. They emailed 1,157 subscribers to the DTB and had a response rate of 14.2% (n=164). The limitations of this research were that they had a low response rate, it only targeted HCPs who had subscribed to the DTB, and was limited to mainly doctors and clinicians rather than a variety of HCPs; thus, limiting the generalisability of the results. Despite this they did uncover some interesting results (DTB, 2010).

Using social media to conduct research provides a unique opportunity to engage a diverse range of people from around the world in a study, quickly and efficiently. Enabling researchers the freedom to explore concepts in an innovative way and people to share their thoughts and perspectives on a subject they are interested in (Woodfield et al., 2013). Traditional research methods have been tried and tested; their long standing use has been refined and guidelines to conduct ethical, safe, reliable and valid data are widely available. Conversely, the use of social media for conducting scientific research is a relatively new phenomenon; any new research done this was will help to highlight the benefits and drawbacks of this research method. Scientists should be willing to move with advances in technology instead of sticking to traditional methods (Murphy et al., 2013).

3.5 Methodology

The following section details the research methods used to conduct this study; describing the design of the questionnaire, the participant inclusion criteria, how the survey was distributed, the ethical approval process, and how anonymity and confidentiality were maintained.

3.5.1 Questionnaire Design

In order to explore HCPs opinions and knowledge of HMs a questionnaire to meet the aims and objectives of this investigation was constructed (Appendix 6); with a combination of open and closed questions to obtain both quantitative and qualitative data. It was designed to be an online, self-completion questionnaire, giving participants the flexibility to complete the survey in their own time (Moore, 2000). An introductory statement was created to advise participants on the purpose of the research and inclusion criteria (Appendix 7). Brent (2014) claims the more questions you ask the less time participants spend on each question. It was important to keep the questionnaire concise as it was anticipated that participants may be deterred from a lengthy survey. For this reason there were only twenty questions split into four sections, estimated to be completed within 10-15 minutes. The first section set out to explore participants' personal use of HMs: looking into the traditional use of HMs amongst families and cultural backgrounds, and HCPs knowledge of HMs for specific ailments. The second section focused on patients' use of HMs, whereby HCPs experience of adverse effects and interactions caused by HMs were investigated. The third section looked at the HCPs' professional opinions of THM; exploring their perceptions of safety and efficacy of HMs, and opinions on the level of education on alternative therapies during their professional training. The final section was designed to evaluate the population demographics, exploring ethnic origins, gender, and occupations.

3.5.2 Participant Inclusion Criteria

The definition of a HCP was explored in section 3.4.1 (p84). After considering the definitions of a HCP and receiving feedback from the De Montfort University (DMU) Ethics committee (Appendix 2A) it was concluded that the term healthcare professionals (HCPs) would be used to refer to those who currently have or previously had interactions with patients dealing with any aspect of patient care. This could include: doctors, consultants, surgeons, nurses, dentists, pharmacists, optometrists, and physiotherapists, herbal medicine practitioners, etc.

The principal requirement for the participant inclusion criteria was that participants had to be a HCP from the UK or having some experience of working in the UK; this was so the results could be generalised to UK HCPs. Undergraduate students currently completing degrees or courses were not directly invited to participate, if the students had training placements or contact with patients their responses were used for the analysis (e.g. pre-registration pharmacists or optometrists or students with clinical placements). There were no other defined parameters for the research sample (i.e. gender, age, or occupation), as this was an exploratory survey to investigate the general perceptions of HCPs on HMs.

3.5.3 Distribution of the Questionnaire

Initially, it was intended that the questionnaire would be posted to HCPs, mainly doctors' surgeries and pharmacies around Birmingham and Leicester (the same cities where the SATMED questionnaires were conducted). However, this raised some doubt about the ethical approval required to send questionnaires to doctors who were contracted with the NHS (see section 3.5.4, p98). Also, the cost of printing and posting the questionnaires, the time consuming process, and low response rate associated with postal questionnaires were outweighing the benefits of conducting the research.

The concept of using SurveyMonkey (an online survey software) and distributing it via social media was discussed amongst the research team and was considered to be a better alternative; as it avoided the cost of printing and posting the questionnaires. As the questionnaire was published online, distribution was not limited to two cities (Birmingham and Leicester); instead participants from across the UK were able to complete the survey. It was anticipated to be a quicker and easier way of distributing the survey to the target audience. The survey was available online for one year from 12th May 2014 -12th May 2015; this gave participants the freedom to complete the survey at their own leisure.

The link to the online survey was emailed to friends and colleagues by the research team, participants were then asked to forward the email to other HCPs they knew. The link was circulated around Lloyds Pharmacy stores around the West Midlands with the approval of the area and regional managers. In addition, it was posted onto the researchers' personal Facebook and Twitter accounts, where friends who were HCPs were asked to complete the survey and share the link on their own profiles too. The link was re-tweeted (shared) by various HCPs and organisations on Twitter including The Guardian Healthcare. The distribution method used led to a snowball sampling technique being adopted as the survey was passed on from person to person.

3.5.4 The Ethical Debate

To satisfy the De Montfort University (DMU) ethical guidelines, any changes to the original PhD proposal which required ethical approval needed to be declared to the DMU ethics committee and approved before the amendments were imposed. An amendment form was completed and submitted for review (Appendix 2A). The ethics committee highlighted several points which needed to be addressed (Appendix 2B). The main concern was the requirement of the National Health Service Research and Development (NHS R&D) approval for the study as it was going to explore the views of HCPs who worked for the NHS.

Contact was made with the NHS R&D team at Leicester City Clinical Commissioning Group (CCG), who initially said the University ethical approval was sufficient for the online distribution of the questionnaires. If the questionnaires were to be posted to doctors surgeries then approval from local CCGs would need to be obtained. The ethical approval for the project was debated amongst the Leicester CCG as there were no guidelines for online surveys with HCPs. Governance Arrangements for Research Ethics Committees (GAfREC), published by the UK Health Departments in May 2011 states, "*Research Ethics Committee (REC) review is not normally required for research involving NHS or social care staff recruited as research participants by virtue of their professional role,*" (NHS Health Research Authority, n.d.). As the online surveys were to be completed by HCPs in their personal time some said NHS ethical approval was not required.

Several correspondences later it was decided that the NHS R&D and Site Specific Information (SSI) forms needed to be completed via the Integrated Research Application System (IRAS) as the team at the Leicester CCG were collectively unsure about how to treat online surveys. SSI forms detail what activities will happen in precise locations and separate forms are required for each CCG; however, as the survey was distributed online the locality of the HCPs could not be forecasted. Despite this it was agreed to complete the process to ensure the study was conducted ethically. The time consuming application process was completed and ethical approval for the study was granted by the Leicester City CCG which provides the Primary Care R&D Service across Leicester, Leicestershire and Rutland (Appendix 2C).

Beninger et al. (2014) identifies the difficulties of applying traditional ethical principles to newly developing methods such as online and social media research. Due to the limited guidance available it proved to be a challenge; but one where valuable lessons were learnt.

3.5.5 Anonymity and Confidentiality

As no personal identifiable data was obtained participants anonymity was ensured throughout the process. The demographic data obtained at the end of the questionnaire was designed to evaluate the types of HCPs who were completing the questionnaire, their age, place of birth and ethnic background. No personal contact details were taken and there was no way of tracing where responses had come from.

The survey was designed and published online via SurveyMonkey. It was a password protected website which only the primary researcher had access to, ensuring all responses were kept confidential. By having all the data password protected the researcher ensured there was no unauthorised access.

3.5.6 Analysis of the Questionnaires

The SurveyMonkey software created graphs for quantitative questions, and enabled the results to be exported; summarising them into a portable document format (pdf) or PowerPoint presentation. Although this software enabled the data to be summarised it did not allow cross comparisons or correlations to be made. For this reason the results were then manually imported into the Statistical Package for the Social Sciences (SPSS) software (version 21), where they were analysed.

3.6 Results and Discussion

A total of 112 respondents attempted the questionnaire, of which 93 were completed. Of the total number of participants who started the questionnaire, 19 withdrew at different stages; 11 participants stopped after question 3, 3 participants left after question 4, 1 stopped after question 9, and the other 3 participants left out the 'about you' section. It was contemplated to discard the incomplete surveys; however, this would mean losing some valuable data. For this reason all responses were included in the analysis and the number (n) of respondents for each question has been highlighted.

3.6.1 Sample Description

The *'about you'* section of the questionnaire was designed to get some background information about the research participants. As 19 participants withdrew by this stage of the survey, these results are from the 93 participants who completed the entire questionnaire.

Age and Gender Profiles

Figure 3-1 summarises the age and gender profile of the research sample: 57% (n=53) were female and 43% (n=40) were male participants. Most of the participants (n=33) were in the 21-30 years age group; nevertheless, there was a range of participants representing the other age groups too. The two participants in the '20 years or under' age group where both optometry students who had clinical placements; thus, their responses were included in this analysis.

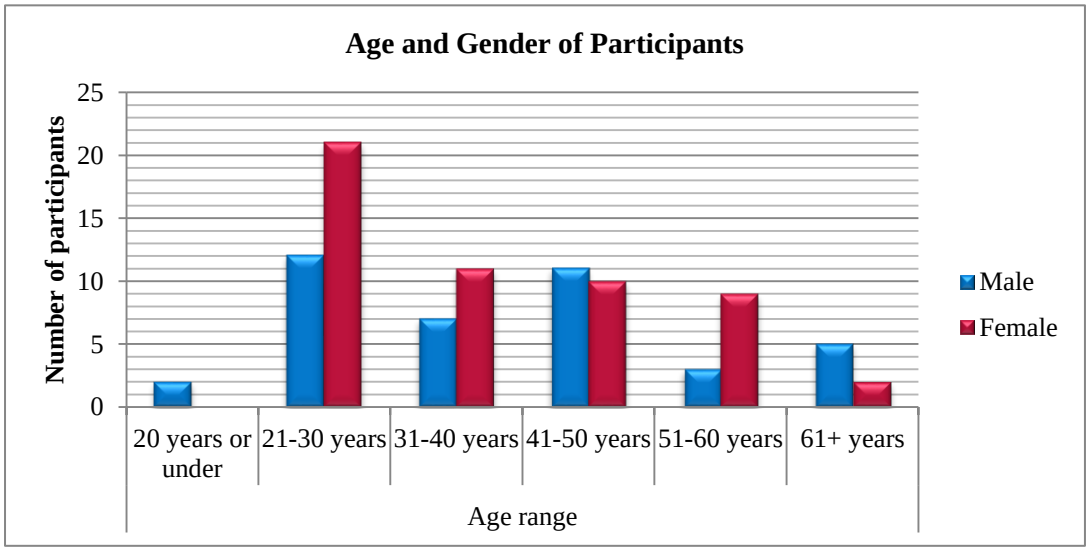


Figure 3-1 Healthcare Professionals' Age and Gender Profiles

Ethnic Origins

The two main ethnic groups participants identified with were White-British (39%, n=36) and Asian/ Asian British-Indian (38%, n=35). Figure 3-2 identifies the proportion of participants from each ethnic background. The 7% (n=6) of participants who selected other claimed to be White-Italian, White-American, White-other, White-mixed, mixed White-British with Black-Caribbean, and Turkish.

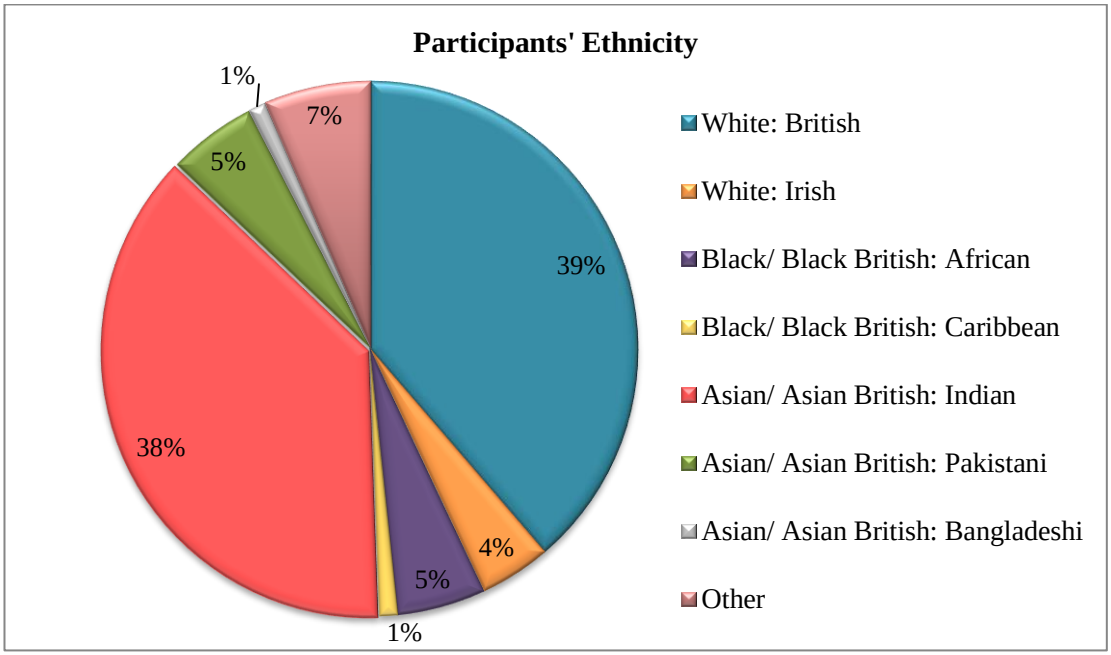


Figure 3-2 Healthcare Professionals' Ethnic Origins

Birthplace & Religion

The majority of participants (81%, n=75) were born in the UK, 8% (n=7) were born in India, 5% (n=5) in Africa and the remaining 6% (n=6) who selected '*other*' said they were born in Albania, Australia, Ireland, Poland, or the USA. Some religions have a strong affiliation with HMs; for example black seed (*Nigella sativa* L.) is claimed to be the cure for all diseases but death according to Islamic beliefs (Ansari and Satish, 2013), while Tulsi (*Ocimum tenuiflorum* L.) represents a goddess in Hinduism (Miller and Miller, 2003). There was a range of participants from each of the religious options presented; 25% Christian, 20% Hindu, 11% Sikh, 10% Muslim, 4% other (other religions listed included Buddhist, Jain, Jewish, and Humanist), while 30% (n=28) of participants claimed to have 'No Religion'. Participants were asked about their birthplace and religion to see if any correlation between their knowledge and use of THM was influenced by their background (this will be explored later in the results section).

Highest Qualification

There was a vast array of qualification listed by participants, including: GCSEs, A-Levels, diploma, bachelor's degree, postgraduate/ clinical diplomas, Bachelor of Dental Surgery (BDS), Fellowship of the Royal College of Surgeons (FRCS), Doctor of Medicine (MD), Masters, Master of Science (MSc), Master of Philosophy (MPhil), Master of Pharmacy (MPharm), Doctor of Pharmacy (PharmD), Member of the Royal College of Psychiatrists (MRCPsych), Bachelor of Medicine and/or Surgery (MBBS), Member of the Royal College of Obstetricians and Gynaecologists (MRCOG), Member of the Royal College of General Practitioners MRCGP, Doctor of Philosophy (PhD), and post-doctorate (All Acronyms, 2015). A large number of participants (46%, n=43) had a Masters Level or equivalent qualification (Figure 3-3). Several pharmacists who had either an MPharm or bachelor's degree in pharmacy stated their highest level of qualification was their post-graduate/clinical diplomas. Two participants selected the '*other*' option, of which one stated '*professional*' and the other '*Fellow of the Society of Biology*.' The high level of qualifications participants have reflects the types of jobs they have, and the range of HCPs who completed this survey.

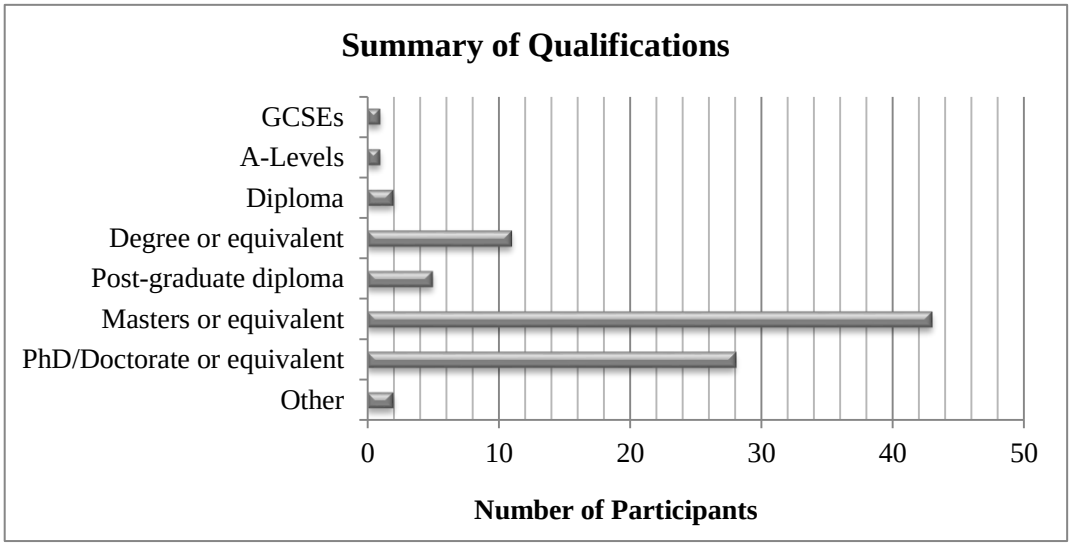


Figure 3-3 Healthcare Professionals' Qualifications

Job Role:

Over 42 different occupations were listed by participants. Table 3-1 outlines the different job roles participants stated. It would not have been possible to recruit the variety of HCPs if traditional research methods such as postal questionnaires were used. If the surveys were posted to pharmacies and GP surgeries the responses would have been limited to community pharmacists, general practitioners, and practice nurses. Due to the survey being published online, it was easier to forward the link to a diverse range of HCPs.

Table 3-1 Summary of Healthcare Professionals' Occupations

Academics:	Medical doctors:	Nurses:	Pharmacy:	Other HCPs:
Biophysicist (1)	Anaesthesia registrar (1)	Hospital nurse (2)	Community Pharmacist Manager (9)	CBT therapist (1)
Lecturer (1)	Consultant physician (2)	Nurse consultant (1)	Clinical / Chief / Lead/ specialist Pharmacists (6)	Dentist (2)
PhD fellowship (2)	General practitioner (8)	Practice nurse (1)	Hospital Pharmacist (3)	Deputy chair BMA (1)
Professor (3)	Non consultant carer grade psychiatrist (1)		Non-Pharmacist manager (1)	Independent public health specialist (1)
Senior lecturer (1)	Foundation year 1 doctor (1)		Pharmacist (23)	Optometrist (1) Optometry student (2)
Clinical research consultant (1)	Specialist registrar in Obstetrics and gynaecology (1)		Pharmacy technician (1)	Renal specialist dietician (1)
Researcher (2)			Pharmacy staff (1)	Speech and language therapist (1)
			Pre-registration Pharmacist (2)	Various/ unspecified (4)

Key: (n = the number of participants identified under each profession).

Do you currently work/ have worked in the UK?

This question was added due to concerns over the survey spreading internationally on social media, as the link was being rapidly circulated. Although the introductory paragraph before starting the questionnaire clearly stated *'for UK healthcare professionals'* there was a risk of respondents not acknowledging this. As this question was added at a later date just 20 (22%) participants who completed the surveys responded; all of whom did confirm they currently did or had worked in the UK.

3.6.2 Healthcare Professionals' Personal Use of Herbal Medicines

Questions 1-4 were designed to explore HCPs personal knowledge and use of HMs. The first three questions were answered by 112 participants who attempted the questionnaire; after which 11 participants withdrew.

Question 1: ***Is traditional herbal medicine part of your family/ cultural background?***

Of the total population who completed this question 45% (n=50) said HMs were part of their family/ cultural background; while, 55% (n=62) said it was not. The demographics of the participants indicated they were from a range of ethnic backgrounds, religions, and countries. A comparison of participants' ethnic origins and whether or not they said HMs were part of their family/cultural background revealed some interesting findings (Figure 3-4). More participants from the SA ethnicities (i.e. Indian, Pakistani, and Bangladeshi backgrounds) claimed the use of HMs was part of their family and cultural background. Whereas, participants from Western cultures (i.e. White: British and Irish) stated HMs were not part of their family or cultural backgrounds.

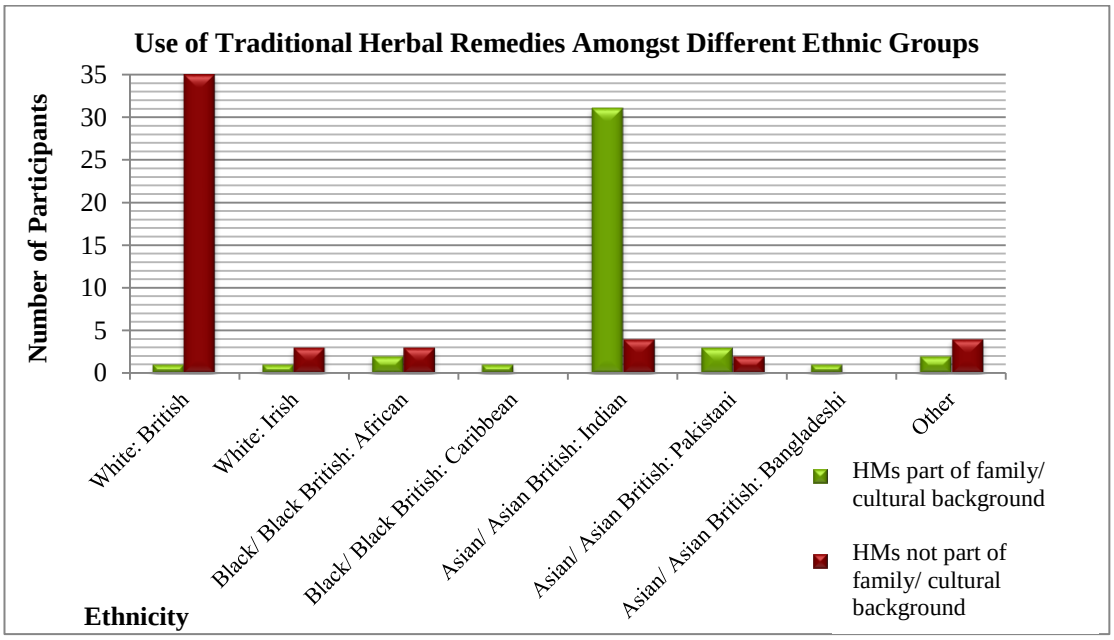


Figure 3-4 The Link Between Ethnicity and Traditional Remedies Being Part of Participants' Cultural Background.

An Analysis of Variance (ANOVA) test was used to see whether or not there was a statistically significant difference between ethnicity and the traditional use of HMs by participants' families. The Null hypothesis would be 'the traditional use of HMs is not linked with ethnicity'; while, the Alternative hypothesis would state 'the traditional use of HMs is associated with ethnicity.' If the significance (Sig) value is less than or equal to 0.05, there is a significant difference between the two variables (Pallant, 2007). The results from the

ANOVA test gave a Sig value of 0.00 which is lower than 0.05 (Table 3-2); thus, a significant difference between the traditional use of HMs and ethnicity can be assumed. For this reason the null hypothesis can be rejected and the alternative hypothesis can be accepted, as a difference between the traditional use of HMs by families in different ethnic groups was statistically significant. Therefore, it can be concluded that the use of THM is associated with ethnicity.

Table 3-2 ANOVA Summary Data for Ethnicity and the Traditional Use of Herbal Remedies

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	14.034	7	2.005	18.938	0.000
Within Groups	8.998	85	0.106		
Total	23.032	92			

Question 2: ***Do you use any plants, herbs or spices for your own health?***
****If 'Yes', what do you use and why?***

The Ipsos MORI report (2008) stated those educated to a degree level or above were generally more likely to use HMs. From the qualifications listed by this research population it is clear participants were highly educated, 68% of participants had qualifications higher than a degree. Despite this, a large proportion of participants (62%, n=70) said they did not use any HMs for their own health. The 38% (n=42) of participants who did claim to use plants, herbs, or spices for their health listed a range of different products (Table 3-3). Numerous remedies for coughs and colds were listed including: honey and lemon, turmeric in milk, Echinacea, tulsi, ginger, and cinnamon. Mint, ginger, cardamom, and cumin were frequently mentioned for improving digestion. Various remedies for skin conditions such as aloe vera, turmeric, and neem were noted. For stress, anxiety and mild depression products such as St John's wort, valerian, and passion flower were documented. The most popular product mentioned was turmeric, cited by 45% of participants (n=19); its uses as an anti-inflammatory and antiseptic for skin infections and wound healing, coughs, colds, and immune boosting properties were detailed. Other products frequently cited included: ginger, cloves, mint, honey, lemon, aloe vera, and herbal sleeping aids. Some products such as fever grass, Sorrel Bitter herbs, nettle tea, and bush tea were identified by just a small number of participants.

Similar to the results from question one, SA participants claimed to use HMs more than any of the other ethnic groups. The responses from participants of SA origin were compared to the other ethnic group participants, and a distinctive difference was seen. Most of the

products listed by SA participants included herbs and spices native to Asia such as ajwain, cardamom, and tulsi. Whereas products listed by the other ethnic groups were native to Western countries for example Agnus castus, camomile, Echinacea, nettles, and valerian. The most popular Western herbal product noted was Echinacea (*Echinacea angustifolia* DC.) which is native to North America (Chevallier, 2007). It has a long standing use in traditional medicine and has been considered as one of the most important medicinal herbs used to treat common cold and infections (Rezaie et al., 2013). Miller (2005) identifies it as "*one of the top-selling herbs of all time*," as numerous commercial products in different pharmaceutical forms are available. The evidence supporting the use for Echinacea, as with most other herbal remedies, is variable (Miller, 2005).

Table 3-3 Summary of the Herbal Remedies Used by Healthcare Professionals

Participant Number:	Herbal Remedies Used:
2	*Rhodiola for energy.*
3	*Fresh mint in tea to help aid digestion.* *Turmeric Powder for upset stomachs.*
4	*Honey, ginger, lemon mixture for dry coughs.*
6	*Nettle tea.*
8	*Turmeric.*
12	*It's not a part of my normal routine but could use one on recommendation depending on the ailment. An example is Turmeric extract for skin inflammation*
16	*Turmeric and salt - antiseptic properties. Cinnamon and ginger - digestive properties Tulsi - digestion and coughs. Cardamom - digestion, infection. Clove -painkiller/tooth pain. Cumin – digestion.*
17	*I use aloe vera from my plant pot for eczema on my hand. I do not like the idea of using chemicals found in creams on my hand which I may then use to pick up food when eating. In addition, I have tried a number of medicinal creams (e.g. steroid creams emollients etc.) which have been ineffective so I wanted to try something different.*
20	*Ginger-sore throats. Turmeric-sore throats. Echinacea-sore throat.*
21	*Turmeric being the main one. If someone has a cold, Turmeric in milk does the trick.*
23	*Tulsi, cinnamon, turmeric, clove, cardamom, aniseed, fennel seeds, fenugreek seeds, mint, honey, nutmeg, okra.* *I use the above to treat cough and cold, diarrhoea, abdominal pains, acidity, toothache, weight loss, indigestion, lower the blood glucose etc.*
26	*Neem tablets and washing products.*
32	*Peppermint tea for indigestion. Honey and lemon for colds.*
35	*Herbal sleeping tablets on occasion.*
44	*Very rarely. In conjunction with OTC medicines I may have used a small amount of ground turmeric in some milk to help with coughs and colds.*
46	*Turmeric for its antiseptic properties, sometimes with a hot chapatti (wholegrain wheat flour based) to act as a heated bandage for sprains. A mixture of chapatti flour and cold water to form a paste to apply on small burns. A mixture of turmeric and honey as a cleansing face mask.*
48	*Mint-for upset tummy, Turmeric as antiseptic.*

Table 3-3 Summary of the Herbal Remedies Used by Healthcare Professionals Continued…

49	*Aloe Vera, Cerissie tea, Fever grass, Sorrel Bitter herbs, Ginger, Garlic, Bush tea.*
50	*Turmeric, Basil.*
51	*I use traditional Chinese herbs for cough related to Aspergillus in my lungs. I tell my consultant I do so. The herbs reduce the mucous, infection and dramatically reduce coughing spasms. The herbs are specifically prescribed for me according to my symptoms.*
53	*Valerian- sleep.*
56	*Camomile tea for relaxation, witch hazel for antiseptic.*
63	*Local honey for hay fever - works amazingly well! Agnus castus for PMS symptoms. I advise patients to try linseed oil for excess GI wind. Some patients report benefits from St John's wort for depression/ anxiety; passion flower for anxiety/ stress.*
64	*Many. Only daily ones are hawthorn and Ginkgo tinctures for circulatory problems. I use natural antiseptics and digestive remedies too.*
70	*Senna is used as a primary constipation therapy.*
74	*Ajwain for indigestion and headaches, cloves for toothache, turmeric.*
81	*Turmeric for infections and skin problems.*
85	*Ginger, tulsi, turmeric.*
86	*Ginger, ground roots for sickness and nausea. Fresh turmeric as a hot drink for cough and sore throat. Dry turmeric powder soaked into cloths for wounds, Clove for toothache. Garlic oil for ear infections. Mint.*
88	*Mentha (calm nerves, eases digestion) lemon, honey, cinnamon (sore throat, colds, allergy, immune system), ginger (nausea, immune system), coconut oil and water (health being, skin).*
90	*Lots- turmeric, ginger, garlic, cinnamon, cumin, fennel, black pepper, honey are the most common that I use here in the UK for various ailments. Sandal wood, red sandal wood, turmeric and honey for skin.*
92	*Ginger tea for cold.*
97	*Ginger for upset stomach.*
101	*Turmeric with hot milk for sore throat.*
102	*Turmeric, mint, cinnamon, pepper, and ginger.*
105	*Echinacea, to maintain immune system. Turmeric, general wellbeing. Aloe Vera skin heath and wound repair.*
108	*My mum thinks having turmeric powder with hot milk helps with coughs.*
112	*Turmeric + Ginger. Provides rapid and effective relief in comparison to conventional medications.*

There is very little research which has explored UK based HCPs personal use of HMs. Generally research focuses on patients' use of HMs, concurrent use of HMs with CWM, or undergraduate students views of HMs (Heuschkel et al., 2002; Freymann et al., 2006; Smith, 2011). The results from this question reveal there are several factors which may influence HCPs decision to use HMs including: traditional use amongst ethnic and family backgrounds, perceived safety and efficacy, and clinical evidence to support the use of HMs. It was clear from some participants' feedback that evidence based medicine was crucial in influencing their decision to take HMs; due to the lack of clinical research to support the use of HMs, HCPs were often reluctant to use them for their own health. While other HCPs revealed the use of HMs has been part of their traditional heritage and the long standing use of HMs is sufficient evidence to support their use.

Question 3: ***Would you prefer to use traditional herbal remedies over conventional Western medicine for minor health problems? E.g. cold/constipation/mild skin irritation?***

The results from this investigation found that 42% (n=47) of participants said they would prefer to use HMs for minor health problems rather than CWM. As reflected by participant 91, a pharmacist, who claimed that, "*using nature's remedies rather than synthetic, has fewer side effects and many more health benefits."* Several references to HMs being natural and therefore safe were noted. In addition, participants claimed the use of HMs had been passed on through generations of the family and therefore they had tried them, when recommended by a family member. There was no correlation between the preference of using HMs with gender, or age; however, similar to previous questions participants from SA ethnic backgrounds generally preferred to use HMs for minor health problems more than other ethnic groups (Figure 3-5).

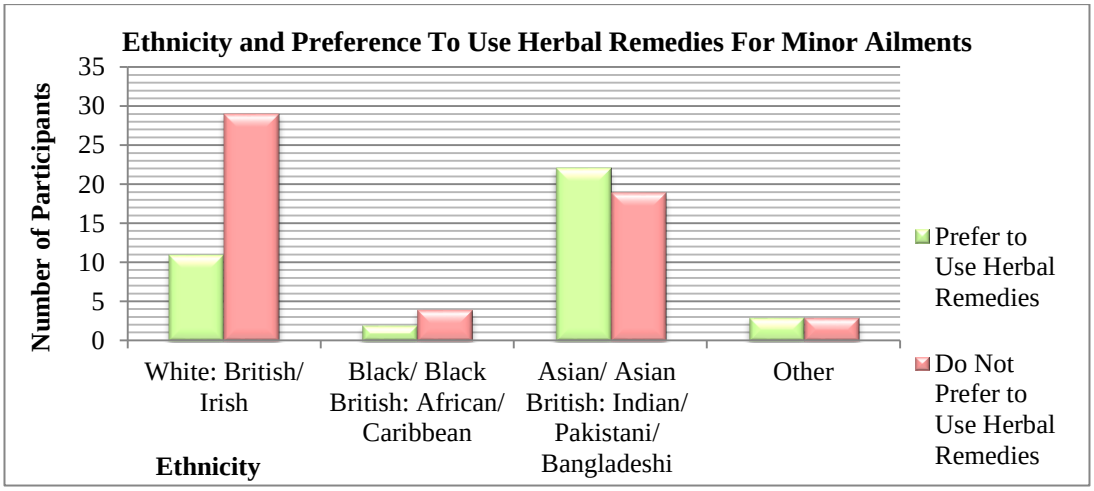


Figure 3-5 Healthcare Professionals' Ethnicity and Preference to Use Herbal Remedies for Minor Ailments

On the other hand, 58% (n=65) of participants claimed they did not prefer to use THM over CWM for minor health problem. The participants forming this research group were all HCPs who were specialists in various sectors of the healthcare framework. Their practice and education is evidence based and their distrust of HMs which lacked scientific validation was evident, as demonstrated by: participant 71 who stated, "*lack of controlled trial data and standardisation of preparations is a crucial weakness in the use of herbal medicines*," and participant 100 who said, "*to be credible more patient and double blind trials need to take place*." Other reasons for not favouring the use HMs for minor health problems included: distrust in natural remedies, the effects of HMs being compared to a placebo, suspicion of unregulated products, and uncertainty of what the HMs actually were (i.e. adulterated species). This was demonstrated by participant 60 who said, "*The difficulties with quality control (specifically in terms of dosage and active ingredients) make treatments with herbal remedies very much a gamble*."

Question 4: ***Can you name any herbal remedies which can be used for the following conditions (arthritis, cough, diabetes, and indigestion)?-If so, please state what could be used.*** *(Question 4 was answered by 101 participants)*

The four conditions selected for this question, arthritis, cough, diabetes, and indigestion, were also used in the SATMED questionnaire (Chapter 2); therefore, it was decided to use the same conditions in this survey. This question was designed to explore HCPs knowledge of HMs for common health problems treated in the UK (WHO 2006; PAGB, 2009; Arthritis Research UK, 2013a). In addition, similarities and differences between the SATMED participants and HCPs knowledge of HMs used for these conditions could be compared.

Less than half of the participants (46%, n=46) did not state any HMs for the conditions listed; these participants either left the question blank or replied with answers such as '*no*,' '*not sure*,' or '*none are useful'*. Research by the DTB found that doctors' knowledge about the clinical aspects of HMs and regulation was poor; of the HCPs who completed their survey 36.2% claimed their knowledge of HMs was '*quiet poor*' while 10.4% said it was '*very poor*' (DTB, 2010). The results from this survey could suggest that the 47% of HCPs who did not state any HMs in this question lacked the knowledge about the clinical benefits of HMs for the conditions stated. On the other hand, it may be argued that just because participants did not list any HMs does not mean they lack the knowledge, but instead they chose not to reply.

The remaining 54% (n=55) of participants did attempt to state HMs for the conditions listed: 31% of the participants stated a HM for just one condition, 15% for two conditions, 24% for three conditions and 30% were able to recall a remedy for all four conditions (Table 3-4). Figure 3-6 illustrates the number of participants who recalled a herbal remedy for each of the conditions; cough (n=37) and indigestion (n=41) had more responses in comparison to arthritis (n=30) and diabetes (n=28). Cough and indigestion are amongst the top ten most common minor ailments treated by doctors and over-the-counter by pharmacists in the UK (PAGB, 2009), which could explain why more HCPs knew about HMs for these conditions in comparison to arthritis and diabetes.

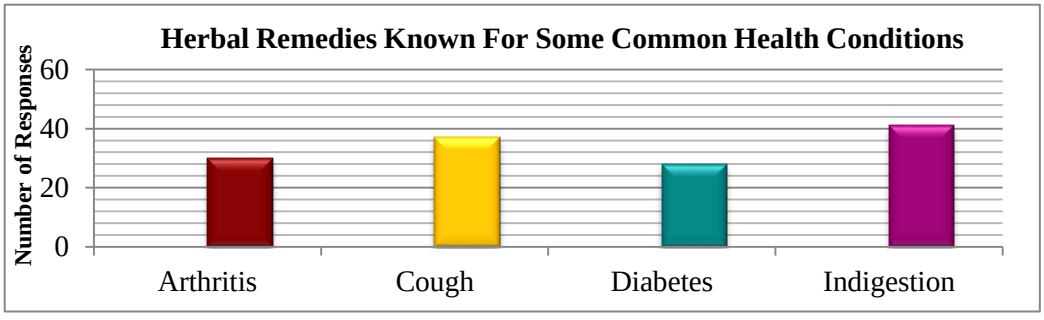


Figure 3-6 Healthcare Professionals' Who Recalled a Herbal Remedy/Remedies for the Conditions Listed

Table 3-4 Summary of Herbal Remedies for Arthritis, Cough, Diabetes, and Indigestion

Arthritis	Cough	Diabetes	Indigestion
Apple cider vinegar (2)	Basil	Aloe vera (2)	Ajwain/ *Trachyspermum ammi* (3)
Avocado with Soybean	Black pepper (2) Black pepper in honey	Bilberry extract (2)	Anise tea Aniseed (3)
Black Currant Oil	Cardamom	Bitter gourd /karela (11)	Cabbage core (contains pepsin)
Boswellia (3)	Coltsfoot	Black seed	Cardamom
Bromelain	Echinacea	Cinnamon (6)	Camomile
Chondroitin	Garlic (2)	Fenugreek (4)	Cloves
Cod liver oil (2)	Ginger (4)	Garlic	Cold milk
Commiphora	Jeevan San Jeevan (an Indian herbal remedy)	Green tea	Corn flour paste
Devils claw (4)	Liquorice (2)	Ginger	Fennel (8)
Eucalyptus oil	Honey (2) Honey in warm milk Honey in hot water with/without lemon (8) Honey, lemon &ginger Honey & lemon/ black pepper	Gurmar	Ginger (6)
Fenugreek seeds	Mallow	Okra (2)	Haajmola/ trifla churan (2)
Garlic	Pineapple juice	Sweet potatoes	Liquorice and coriander
Ginger (4)	Poppy	Tulsi (basil)	Milk thistle
Glucosamine (5)	Squill	Turmeric	Mint (5)
Rosehip (2)	Tea		Peppermint (1) Peppermint oil Peppermint tea (3)
Sesame seed oil	Thyme (3)		Plantain and green bananas for ulcers
Tulsi	Tulsi (2)		Senna
Turmeric (6)	Turmeric (10) Turmeric powder in milk		Suva (Dill seeds)
Willow bark = aspirin/salicylates (3)			Turmeric
Wintergreen (topically)			

Arthritis Research UK (2012) revealed that approximately 10 million people seek advice from their GP about arthritis; it is a condition which can affect all ages. Arthritis Research UK (2012) have acknowledged that sufferers will be tempted to try alternative therapies and have created a report based on randomised controlled trials which have tested the effectiveness and safety of some common HMs used for rheumatoid and osteoarthritis. It has summarised the safety and efficacy of several products including: fish oil, borage seed oil, evening primrose oil, Cat's claw, and flaxseed oil for rheumatoid arthritis; Indian frankincense, ginger, Devil's claw, willow bark, glucosamine, and chondroitin for osteoarthritis. Each product was given an efficacy score between 1-5 depending on the reported improvement in pain, movement, and general wellbeing; 1 correlated to no or very little evidence, while 5 represented data where there was consistent evidence across several studies. A traffic light system was used to code the safety profile, green represented minor/ infrequent side-effects reported, amber signified commonly reported side-effects (even minor ones) or more serious effects, and red illustrated serious side effects reported. The full report is available for the general public to access to help make more informed decisions about herbal remedies to help with their condition (Arthritis Research UK, 2012). The most common HMs documented for arthritis by participants in this survey were: turmeric, glucosamine, ginger, Devils' claw, cod liver oil, rosehip, apple cider vinegar, and willow bark (Table 3-4). Many of these remedies were similar to those listed in the Arthritis Research UK (2012) report '*Complementary and alternative medicines for the treatment of rheumatoid arthritis, osteoarthritis and fibromyalgia.*' Several remedies such as turmeric, ginger, and sesame seed oil were similar to responses listed by the SATMED survey participants. The key difference noted was that the participants in this survey mentioned products such as glucosamine, rosehip, Boswellia and willow bark, which none of the SATMED participants identified. Boswellia (also known as Indian frankincense, *Boswellia serrate* Roxb. ex Colebr.) is famous for its use in the traditional Indian medical system of Ayurveda (Siddiqui, 2011). The resin from the trees has an anti-inflammatory action as it is rich in mono/di/tri-terpenes and pentacyclic triterpenic acids i.e. β-boswellic acid which inhibit pro-inflammatory enzymes (Sontakke et al., 2007).

Research by the Royal Pharmaceutical Society (2014) found that common ailments such as coughs and colds cost the NHS £1.1 billion a year, when patients are treated by their GPs or Accident and Emergency. The HMs mentioned for cough included turmeric, tulsi, honey (with or without lemon, black pepper, ginger, in hot water), and liquorice. HMs such as poppy, squill, coltsfoot, mallow, and thyme were mentioned specifically by White-British/ Irish participants. Mallow (Marshmallow, *Althaea officinalis* L.) is a traditional European herb; that has demulcent properties which help sooth a cough (Chevallier, 2007). In January

2014 a new product was introduced by Omega Pharma called Bronchostop, a cough syrup which combines Marshmallow root and thyme extracts (Buttercup Bronchostop, 2014). It is marketed as a traditional herbal medicine as the evidence for its efficacy was based on its traditional use. The development, marketing, and success of new HMs suggests consumers are interested in trying natural remedies; as implied by the sales figures of Bronchostop which were reported to the value of £4.36 million since being launched in 2014 (OTC bulletin, February 2015). Those participants who did not support the use of HMs for the treatment of cough made references to HMs having a placebo effect or not having any effect on treating a cough at all, like participant 62 who claimed that, *"none are useful (neither are any conventional medicines),"* while, participant 51 stated she only used medicines *"on prescription from my practitioner"* for a cough.

For indigestion peppermint, fennel, mint, aniseed, and ajwain were the most popular HMs mentioned. Participant 85 listed senna as being useful for indigestion; however, it is licensed for use as a stimulant laxative for constipation, which can cause abdominal cramping as a side-effect, making indigestion feel worse (Chevallier, 2007). Although there is no evidence to support the use of senna for indigestion it may be something the participant tried and found useful.

WHO (2006) estimated that in 2000, 171 million people around the world had diabetes and they predicted it would more than double by 2030 to 366 million people; however, in 2014 an estimated 422 million adults were living with diabetes (WHO, 2016). There are currently 3.3 million people diagnosed with diabetes in the UK, a condition which can affect any age, cannot be cured, and in most cases requires medical treatment (Diabetes UK, 2015). Diabetes can have serious health consequences if not managed correctly. The most popular HMs mentioned for diabetes were karela, cinnamon, fenugreek, aloe vera, and okra. Other products listed by the HCPs included bilberry, black seed, sweet potato, and gurmar. Gurmar (*Gymnema Sylvestre* (Retz.) R. Br. ex Sm.) in Hindi translates to 'destroyer of sugar' and it has been used traditionally to treat diabetes and symptoms associated with diabetes such as urinary frequency (Saneja et al., 2010).

Many similarities between the products/ HMs recalled in this survey and the SATMED questionnaire were noted; i.e. turmeric, ginger, tulsi, garlic, mint, and aniseed were mentioned frequently by participants in both surveys. Whereas, products such as rosehip, Marshmallow, willow bark and Devil's claw were only identified by participants in the HCPs survey. Unlike the SATMED questionnaire which was for SA participants only, this survey was open to participants from all ethnicities which has enabled more varied responses to be collected. Participants from the non SA ethnic groups listed more Western herbals such

as coltsfoot (*Tussilago farfara* L.) and nettles (*Urtica diocia* L.) which are native to Europe (Chevallier, 2007). A difference in knowledge of HMs was also identified between the ethnic groups, as more SA participants (n=28) were able to list HMs for the conditions than White British/ Irish participants (n=13); this suggests that SA HCPs had more knowledge of HMs for the conditions listed compared to HCPs from other ethnicities.

Overall the results from question 4 have revealed a diverse range of HMs for arthritis, cough, diabetes, and indigestion known by the HCPs (Table 3-4), exemplifying the wealth of knowledge HCPs possess.

3.6.3 Patients' Use of Herbal Medicines

This section of the survey tried to establish whether or not patients reported their use of HMs to their HCPs, how often HCPs came across patients using THM, and HCPs experience and knowledge of adverse effects and interaction caused by the consumption of HMs. This analysis represents the results of 98 participants who completed this section.

Question 5: ***Do you currently have contact with patients?***

This question was added shortly after the survey was launched; as after reviewing the initial responses it was decided to assess whether or not HCPs were actually dealing with patients. This was to determine how many of the respondents were actively practicing their profession. As the question was added later, 11 participants had missed the question. Of those who did complete the question 91% (n=79) said they were in contact with patients, while just 9% (n=8) were not. Participants who were not in contact with patients were generally working in academia, research, or retired; they completed the survey based on their previous experience while working with patients. In addition, some HCPs no longer had regular contact with patients but were involved in different aspects of patient care for example superintendent pharmacists responsible for the overall management of professional services across pharmacies.

Question 6: ***Do your patients tell you about any herbal medicines they use?***

As many as 52% of patients in the UK have tried some form of complementary therapy at some point, with HMs reported as the most common form used (Posadzki et al., 2012). One third of HCPs (33%, n=32) who answered this question said their patients did not tell them

about any HMs they used. A small number of participants (2%, n=2) selected the '*not applicable*' option as they did not have contact with patients, so could not answer the question. Newall et al. (1996) claims that doctors are becoming increasingly aware of their patients' use of HMs. This is supported by the results of this survey which revealed that 65% (n=64) of HCPs said their patients did tell them about their use of HMs. Practitioners of CWM have not always accepted the use of HMs; however, there appears to be a change in the attitude of Western HCPs to complementary therapies (Vickers, 2000). In order to build trust with patients, so patients feel comfortable telling HCPs about their use of HMs, HCPs must keep an open mind and be approachable (GMC, 2009).

Vickers (1994) identified that there is an increasing use of complementary therapies across Europe, and doctors should routinely include questions about complementary therapies when taking a patients history. Upon reflection of this survey, a question about whether or not HCPs asked patients about their use of HMs should have been included; to see if HCPs were proactive in finding out about their patients' use of HMs. Heuschkel et al. (2002) looked at the use of HMs in children and young adults with inflammatory bowel disease and found that just 8% of doctors initiated a discussion about CAM. If patients do not tell their HCPs about their use of HMs it can have serious consequences, such as patients delaying seeking medical attention which could exacerbate their condition (Pharmaceutical Press, 2013). HCPs should be encouraged to routinely ask patients about their use of HMs (Heuschkel et al., 2002).

Question 7: ***How often do you come across patients who take herbal medicines alongside their prescription medicines?***

When the initial version of the questionnaire was submitted for review to the DMU ethics committee this question was created using the likert scale: 'Very Frequently, Frequently, Occasionally, Rarely, Never.' The ethics panel review of the questionnaire led them to ask, '*how frequently is frequently?'* They suggested modifying the scale to justify the frequency of reporting; thus the terms 'Daily, Weekly, Monthly, Annually, and Never' were used. Figure 3-7 reveals that most HCPs did come across patients who were using HMs alongside their prescription medication. Very few HCPs (3%, n=3) claimed they met patients who told them about their use of HMs daily (this would be equal to the 'very frequent' option on the original likert scale).The majority of HCPs (35%, n=34) said patients reported their use of HMs 'monthly', an equal proportion (22%, n=22) selected 'weekly' or 'annually'. Those HCPs who disclosed that patients never reported their use of HMs (17%, n=17), most worked in academia or research, some were pharmacists in managerial or office based roles (i.e. chief or clinical research pharmacists), optometry students, and a dentist.

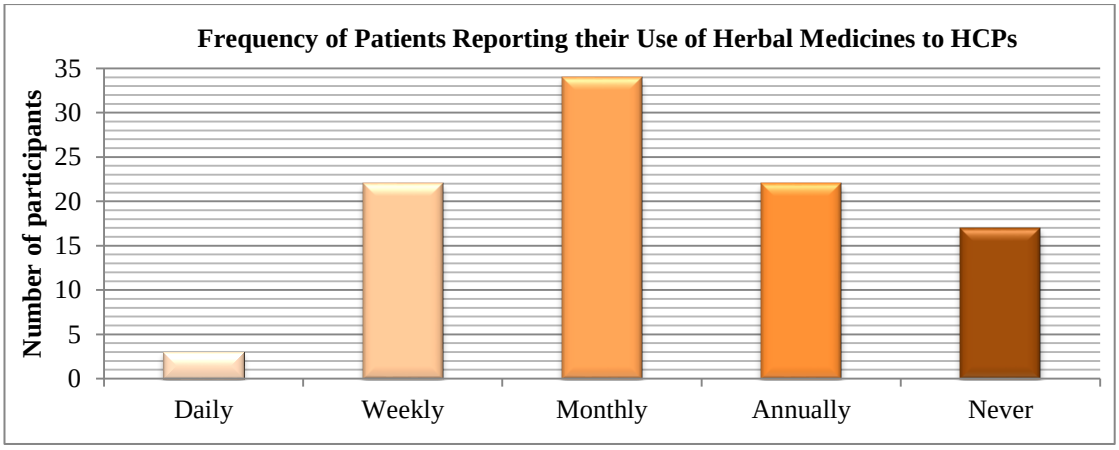


Figure 3-7 Frequency of Patients Reporting their Use of Herbal Medicines to HCPs

De Smet et al. (1997) claim patients may feel reluctant to tell their HCPs about their use of HMs; this was also observed in the results from the SATMED questionnaire, where participants did not want to tell their HCPs about their use of THM. The SATMED questionnaire participants revealed they were scared of being treated differently or judged by their HCPs. HCPs have the opportunity to build a relationship and trust with their patients. During consultations if patients feel they can trust their HCP they may feel more inclined to disclose their use of HMs. Patients may not think that reporting their use of HMs is important, due to the common misconception that, 'herbal remedies are natural and therefore safe' (Ipsos MORI, 2008; Shaw et al., 2012). In some situations, such as before surgery, it is vital for HCPs to know whether or not patients take HMs as there could be complications if not disclosed (Ang-Lee et al., 2001). Overall, the results from this question suggest that more HCPs claimed that they were informed about their patients' use of HMs than those who were not.

Question 8: ***Have you encountered any adverse effects caused by the consumption of herbal medicines?***

Adverse effects from HMs could be anything from a minor side-effect, such as local irritation from using witch hazel for skin conditions, to life threatening reactions like cancer or hepatotoxicity caused by comfrey (Pharmaceutical Press, 2013); 31% (n=30) of HCPs in this survey claimed to have encountered an adverse drug reaction (ADR) associated with HMs (Table 3-5). An example of a serious herbal-ADR caused by the consumption of garlic supplements was identified by participant 15, an anaesthetic registrar, whereby the patient had impaired blood coagulation which caused severe blood loss during surgery. It has been suggested that garlic can have an anti-platelet effect similar to that of low-dose aspirin; therefore, if taken with other blood thinning drugs there is an increased risk of bleeding

(Williamson et al., 2013). Participant 22 (a medicines information pharmacist) recalled a patient with deranged liver functions after taking Kava kava (*Piper methysticum* G.Frost.); a product which is now prohibited in the UK and many other countries due to the fatal hepatotoxic effects associated with its use (Mann and Andrews, 2007). In 2006, 79 reports of liver damage associated with the use of Kava were reported to the MHRA (Pharmaceutical Press, 2013), which eventually led to it being banned in the UK.

There are several well documented adverse effects caused by the consumption of HMs, including: heartburn caused by peppermint, hypoglycaemia caused by karela or fenugreek, and renal failure or cancer caused by *Aristolochia* species (Newall et al., 1996; Shaw, 2010). Although peppermint is considered to be a HM it is one of the few products prescribed by UK HCPs for abdominal colic or irritable bowel syndrome (BNF July, 2015). As it is regulated and its efficacy has been clinically tested, the ADRs associated with its use are well known i.e. as it's an irritant it can cause hypersensitivity reactions such as headache, bradycardia, and muscle tremor (Pharmaceutical Press, 2013). However, with most other HMs there is limited knowledge and information available on the ADRs associated with their use, making it imperative to conduct more research on the safety and efficacy of HMs.

Shaw (2010) claims that adverse effects from HMs may be due to the unregulated use of HMs, poor quality of herbals, adulterated or contaminated herbs, or the wrong or misidentified herbs being used. Participant 59 also listed several reasons why patients may experience adverse effects after consuming HMs, such as *"contaminated products due to poor quality controls"* and *"adverse synergistic effects with existing prescription drugs."* Several references to ADRs caused by Chinese herbal medicines which contain steroid contamination were also noted by participants 49, 72 and 109.

Pharmacovigilance involves monitoring drug safety and adverse effects. WHO (2015) defines pharmacovigilance as, "*science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other drug-related problem*." It begins during clinical trials and continues once the drug is available on the market (Sarker et al., 2015). Although, it is focused on conventional medicines the same principles can be applied to HMs (Barnes, 2003). The Medicines and Healthcare products Regulatory Agency (MHRA) established the 'yellow card scheme' (YCS) to monitor the safety of all healthcare products in the UK, including HMs (MHRA, 2014a). Until 1996 the YCS only collected ADR information on licensed HMs; however, in October 1996 this was changed to include all HMs (Mann and Andrews, 2007). Shaw et al. (2012) claims that as the use of HMs has increased, the reports of adverse effects should have also risen; despite this, the number of reports remains low (Barnes, 2003). This was also found in the results of this survey

whereby 69% (n=68) of HCPs said they had not come across any adverse effects caused by the consumption of HMs. In 2001, 70 reports of herbal-ADRs were received by the MHRA, this formed just 0.4% of the total number or reports received in 2001 (Mann and Andrews, 2007). The latest MHRA Annual Report and Accounts 2014/15 reveals that 14,836 ADRs were reported, a 12.5% increase in the last three years; however, it does not specify the number of herbal-ADRs reported (MHRA, 2014b). The Herbal Medicines Advisory Committee Annual Reports 2014 identified that 51 reports of ADRs from herbal medicinal products (excluding food, cosmetics, and homeopathic products) were received via the YCS in 2014 (MHRA, 2014c).

Unlike CWM which have undergone clinical trials and extensive research to document their safety and efficacy profiles, often the only evidence for the safety and efficacy of HMs is their long standing use throughout history (De Smet et al., 1997). Traditional knowledge of HMs is not well documented, let alone the adverse effects which may be associated. In addition, peoples' lack of knowledge of the adverse effects caused by HMs could have an important role in the severe underreporting of adverse reactions caused by HMs. Educating the public about potential herbal-ADRs caused by HMs could help people use them safely and reinforce the importance of reporting via the YCS (Pharmaceutical Press, 2013). The need to improve pharmacovigilance of HMs to ensure people use them safely is of paramount importance (Barnes et al., 1998).

Table 3-5 Adverse Effects Encountered From Patients Taking Herbal Medicines

Participant Number:	Adverse Effects From Taking Traditional Herbal Remedies:
2	*Loose stools, digestive problems, nausea, dizziness, raised liver enzymes.*
15	*Impaired coagulation leading to excess blood loss at surgery - patient taking garlic supplements.* *Excess liquorice consumption has been reported to cause malignant hypertension.*
20	*Hypoglycaemia with karela.*
22	*Deranged liver function with Kava kava.* *Collapse with multi-constituent product.* *Organ failure in baby from unidentified Asian medicine ....to name a few.*
33	*When I worked in Oncology there were a number of occasions when ladies being treated with trastuzumab for breast cancer asked about whether there was a problem if they were taking a herbal product. The answer was always possibly and in one case the patient taking Hypericum had her dose delayed until she had stopped taking it.*
36	*Renal impairment.*
44	*A number of years ago, i was informed by a colleague of a patient who had been admitted with liver impairment thought to be caused by an Indian herbal remedy.*
49	*Only Chinese herbal medicines that have potent steroids in.*
56	*Not so much herbal but a patient using green vegetable juices for cancer; but, also taking warfarin and ended up with nose bleeds.*
57	*Digestion issues.*
59	*Many adverse reactions due to:* *a) contaminated products due to poor quality controls* *b) deliberately adulterated products to give an effect* *c) adverse synergistic effects with existing prescription drugs* *d) side effects of herbal medicines.*
60	*Malignant Neuroleptic Syndrome.* *Serotonergic Syndrome.*
61	*Liver damage due to use of TCM herbal remedy for eczema.*
62	*Many side effects have been described in literature and in MHRA warnings.*
72	*Renal failure after use of an aphrodisiac steroids in skin cream leading to skin thinning.*
75	*Severe GI bleed resulting in prolonged ICU admission, herbal medicine was for memory (not exactly sure as it has been some time since) but was not gingko.*
79	*Use of garlic.*
82	*Unsure whether traditional remedies were the causative item but dramatic increase in blood pressure for heart failure patient when taking both traditional and Western medicines , but this was about 10 years ago.*
100	*Urticaria.*
109	*Many years ago a patient taking a Chinese tea remedy which had steroids in it.*

Question 9: ***Are you aware of any interactions between herbal medicines and conventional Western medicines?***

Over a third of participants (37%, n=36) said they did not know of any interactions between HMs and CWM; while, the majority of participants (63%, n=62) said they were aware of interactions. Those participants who said they knew of interactions between HMs and CWM, 56% (n=55) named at least one interaction (Table 3-6). The most common herbal product known to interact, described by 37% (n=33) of participants, was St Johns Wort (SJW). SJW is one of the most well-known herbal remedies which has been used to treat mild depression, anxiety, sleep disorder, seasonal affective disorder, toothache, pain, gastritis, stomach ulcers, and aid local wound healing (Chevallier, 2007). Due to the numerous interactions with CWM it has been extensively researched. There are several active constituents in SJW responsible for its diverse medical uses including flavonoids, hypericin, and hyperforin (Williamson et al., 2013). SJW is known to affect a number of the cytochrome (CYP) P450 enzymes which accounts for the range of interactions associated with it. It has an enzyme inducing effect on the CYP 3A4, CYP 219 and CYP 2E1 isoenzymes. The role of these isoenzymes on drug distribution and metabolism is speeded up rendering other drugs less or ineffective; this causes SJW to interact with drugs such as anti-epileptics (carbamazepine and phenytoin), contraceptive pills, ciclosporin, theophylline, antidepressants – monoamine oxidase inhibitors (MAOIs) and selective serotonin reuptake inhibitors (SSRIs), and warfarin (Henderson et al., 2002; Williamson et al., 2013). SJW can also affect the transporter protein P-glycoprotein, which has an essential role in drug absorption from the intestine, and distribution, acting as a drug efflux pump (Gurley et al., 2008). One of the main drugs affected by this mechanism is digoxin, a drug which has a narrow therapeutic index; therefore, small variations in blood concentrations can lead to toxicity or a sub therapeutic dose (Currie et al., 2011). There are several studies which demonstrate that the induction of the P-glycoprotein in the intestine by SJW leads to reduced absorption of digoxin (Hennessy et al., 2002); hence reducing the concentration of digoxin in the blood rendering the drug ineffective. Other drugs affected by this include ciclosporin and fexofenadine (Gurley et al., 2008).

Participants also mentioned the interactions between warfarin and several herbal remedies such as ginseng, gingko, garlic, and cranberry juice. It has been reported that these substances can impair platelet aggregation, therefore effect blood coagulation. The International Normalised Ratio (INR) is a measure of the blood coagulation, which should be between 2-3 depending on the condition for which warfarin is initiated (BNF, 2015). A high INR means the blood is thinner so takes longer to clot, increasing the risk of bleeding; while a low INR means the blood clots too quickly. As these HMs reduce platelet aggregation, if

taken with warfarin they can also increase the risk of bleeding. One study claimed that ginseng reduced the effectiveness of warfarin; however, the pharmacological mechanism remains unclear (Yuan et al., 2004). Reports that cranberry juice inhibited the metabolism of warfarin therefore reducing its clearance from the body and increasing its effects were documented by the Committee of Safety of Medicines /MHRA when several incidents of increased INR and bleeding were reported (CSM/MHRA 2003). A study in the UK which looked at the pharmaceutical care plans of 631 patients in an outpatient anticoagulant clinic found that 170 patients were using some form of HM at the same time as taking warfarin; of these patients 58% were taking something which could potentially interact with warfarin. The authors concluded that by taking a full drug history from patients, potential interactions between HMs and warfarin could be avoided (Ramsay et al., 2005). Fugh-Berman and Ernst (2001) say those taking anticoagulants especially coumarins should generally be advised to avoid taking any HMs.

As the practice of polypharmacy, whereby multiple drugs are consumed concurrently is becoming more common (Edwards et al., 2012), the risk of interactions between HMs and CWM needs to be considered. Furthermore, HCPs need to be more aware about potential interactions which could affect their patients' health. Although there are some valuable texts which summarise herb-drug interactions published (e.g. Stockley's Herbal Medicines Interactions - Williamson et al., 2013), such books are not readily available to HCPs. As identified by participants 20, 59, and 67 there are a variety of sources available to determine herb-drug interactions; however due to the lack of research on many HMs there may be limited evidence available to inform and guide HCPs.

Table 3-6 Interactions between Herbal and Conventional Western Medicines Identified by Healthcare Professionals

Participant Number:	Interactions between Traditional Herbal Medicines and Conventional Western Medicines:
2	*St John's wort with contraceptives and anti-depressants (SSRI's and MAOI's).* *Dan shen with anticoagulants.*
10	*St John's wort interacts with many drugs as it is a cytochrome P450 3AE inducer; for example, oral contraceptives, warfarin, and simvastatin.*
12	*I'm aware of a lot of interactions between herbal medicines and P-glycoprotein and cytochrome enzymes, which can in turn affect drugs that are dependent on them. St John's wort is an example of a herbal medicine that can produce such interactions.*
15	*Too many to list- St. John's wort & SSRIs can cause serotonin syndrome. Garlic, ginkgo & ginseng in combination with fish oil can cause impaired coagulation /blood pressure.*
16	*St. John's wort and warfarin.*
17	*While working as a medicines information pharmacist in secondary care, I formulated a table of interactions between warfarin and a large variety of herbal/traditional compounds.*
18	*St. John's Wort and various other medications, including some anti-retrovirals.*
20	*I refer to Stockley-particularly for St. John's wort.*
22	*INR changes Enzyme induction Additive side effects.*
24	*St. John's wort.*
25	*St John's wort interacts with CYP450 liver metabolised drugs.*
26	*St. John's wort and warfarin.*
30	*Ginseng known to interact as well as St. John's wort with antiretrovirals for HIV.* *Aloe vera reduces drug absorption of many drugs Maca can interact as it's an enzyme inducer.*
32	*St John's wort and a plethora of Western medicines.* *Some questions about the use of antioxidants alongside chemotherapy or targeted therapies.* *Plenty of theoretical interactions e.g. aloe vera juice speeding up the GI tract and possibly causing a reduction in absorbed medications.*
34	*St. John's wort.*
36	*Hepatic enzyme inhibition.*
40	*Cranberry juice and warfarin.*
42	*St John's wort is widely documented.*
48	*St. John's wort with levongesterel.*
53	*St John's wort and P450 interactions.*
56	*Many potential interactions between St John's wort and various medications.* *Also concern with many herbal remedies for renal patients as most herbal remedies filtered via kidneys e.g. liquorice/nettle etc.*
57	*The usual ones given by the MHRA, NIH, etc.*
58	*SSRI and evening primrose.*
59	*Yes, well documented in the literature, e.g.* *http://www.ncbi.nlm.nih.gov/pubmed/10953464* *http://www.sciencedirect.com/science/article/pii/S0024320505012452* *http://archinte.jamanetwork.com/article.aspx?articleid=522673* *http://www.sciencedirect.com/science/article/pii/S1043661810000915*

Table 3-6 Interactions between Herbal and Conventional Western Medicines Identified by Healthcare Professionals Continued…

	and many more.
60	*St John's Wort with SSRI, MAO-I and neuroleptics.*
61	*Enzyme induction e.g. Contraceptive pill failure due to use of St John's wort.* *Especially CYP450 - warfarin potentiation*
62	*Many have been described in the literature.*
63	*St John's wort interacts with lots of things e.g. warfarin, tyramine containing foods. Gingko biloba with warfarin.* *Ginseng with aspirin, clopidogrel, and warfarin.*
64	*I would always check for known or possible interactions and be very careful with things like warfarin.*
65	*Too many to describe.*
66	*St John's Wort interacts with many medicines. There are too many other interactions to list here. See standard pharmacology and pharmacognosy texts.*
67	*I have a book on the subject. Herb-Drug interactions in oncology by Cassileth, Yeung and Gubili, Memorial Sloan-Kettering Cancer Centre People's Medical Publishing House, Shelton Connecticut.*
68	*St John's wort.*
69	*St John's wort interacts with many drugs, e.g. warfarin.*
70	*The basics like St John's wort as an enzyme inducer etc.*
71	*St John's wort interacts with many conventional pharmaceuticals, including statins, beta blockers and antiretroviral drugs.*
72	*St John's wort and anticoagulants.*
73	*But only through the literature. It is always difficult to relate cause and effect in individual cases.*
74	*Garlic and anti-coagulants.* *St John's wort and many medicines.*
75	*St. John's wort, Echinacea, gingko, garlic, red yeast rice.*
77	*Mainly the pill.*
79	*St John's Wort especially.*
81	*St John's Wort.*
82	*Depends if St John's wort is classed as traditional or not!*
84	*St John's wort and warfarin.*
87	*St John's wort etc.*
88	*Ginseng and blood pressure medications.* *Warfarin and high consumption of green vegetables (iron content).*
90	*Saint John's wort and various hormonal medications.*
92	*St. John's wort and warfarin.*
93	*Some augment the actions of oestrogen in HRT.*
100	*Need to avoid with some anti-hypertensive medicines.*
103	*St John's Wort and antidepressants.* *Ginkgo Biloba with various drugs*
104	*St John's wort can interfere with Western antidepressants.*
110	*St John's Wort, Black Cohosh, EPO/GLA, Saw palmetto.*
111	*St John's Wort interacts with contraceptive pill.*

3.6.4 Professional Opinions of Herbal Medicines

The final part of the questionnaire aimed to investigate HCPs professional opinions on the safety and efficacy of HMs. The literature search around this topic revealed that there was limited information available about how much information is passed on to undergraduate HCPs during their courses (Smith, 2011); for this reason participants were asked whether or not they received sufficient education on HMs during their training. This section was completed by 96 participants.

Question 10: ***Do you think the use of plants, herbs and spices is effective to prevent or treat various health conditions?***

Two thirds of the participants (66%, n=63) said they thought HMs were effective. Participants commented on how the long standing use of HMs throughout history was an important factor in determining the efficacy of HMs; as demonstrated by participant 42 (a pharmacist) who stated, *"Pharmacy is generally sceptical about the use of traditional herbal medicines because of lack of scientific studies but, in my opinion, traditional herbal medicines can be very effective in non-Western countries where there is a tradition for this. If something has been used for centuries and works then it works."* In addition, participant 36 (a consultant physician) claimed, "*I am sure that there is some evidence of benefit (even if placebo effect) for some herbal medicines - but I do not see them as a replacement for conventional medicine."* Despite the limited evidence to support the health claims associated with HMs the transmission of this knowledge throughout time has been used an indicator for the efficacy of HMs.

The remaining third of participants (34%, n=33) who did not think HMs were effective or any better than a placebo, made comments regarding the lack of evidence to prove the efficacy of HMs. The emphasis on the lack of reliable scientific evidence to support the use of HMs, was illustrated by participant 62 who said, "*If you look at NCCAM's herbs at a glance (http://nccam.nih.gov/health/herbsataglance.htm) you will see that there is not a single herbal preparation for which there is good evidence for efficacy, even according to this pro- CAM organisation. They originated in a time when anecdote rather than proper testing sufficed; there is no excuse for pharmacies selling things that are either unproven or disproved."*

Although there were mixed reviews about the effectiveness of HMs, some participants suggested that HMs were used to supplement CWM, especially in patients with cancer and HIV. Participant 63 (a GP) said, *"Western medicine alone is not enough to treat someone*

holistically. Additional approaches are required, including herbal medicines and complementary therapies e.g. art therapy, hypnotherapy. Many issues are stress related or exacerbated by stress and these unfortunately are not dealt with adequately." This is supported by findings from Awodele et al. (2012) who uncovered that 41% (n=123) of participants in their survey of Nigerian HCPs thought that HMs were effective for chronic conditions; although none thought that HMs alone could be used to treat a patient. Subscribers to the DTB were asked to complete a survey about HMs, of the 164 respondents who were asked if they thought HMs were helpful: 22.2% (n=36) said '*rarely or never*' while 75.3% (n=122) said HMs were helpful '*in some circumstances*' (DTB, 2010).

It is well known that many of the HMs used are based on their traditional reputation rather than clinical evidence (Newall et al., 1996). In addition, many HMs may be used in their crude form, rather than in pharmaceutical formulations making it difficult to determine the safety and efficacy of HMs due to the vast range of formulations and irregular doses. The emphasis on evidence based medicines requires validated scientific evidence including randomised controlled trials which prove the efficacy of drugs; however, this information is not available for most HMs (Pharmaceutical Press, 2013). More research on HMs is now being conducted to validate the efficacy of HMs. For example, the wealth of literature available on SJW which has provided useful evidence to support the traditional health claims associated with its use. The vast amount of information available on SJW has even led some people to question whether it is still considered a HM or a CWM as stated by participant 82, *"depends if St John's wort is classed as traditional or not!"*

The results for question 10 demonstrate the different views HCPs had regarding the efficacy of HMs. A larger number of participants agreed that HMs were effective for some health conditions; however, there were still some reservations regarding the perceived efficacy of HMs due to the lack of clinical data to support their use.

Question 11: ***Do you think the use of plants, herbs and spices is safe?***

One of the reasons for the popularity of HMs is the belief that they are natural and therefore safe (Shaw et al., 2012); similar views were shared by 58% (n=56) of participants in this survey who thought HMs were safe. Participant 42 (a pharmacist) who agreed HMs were safe stated, *"conventional Western medicines are just as harmful as traditional herbal medicines if used improperly and in excess."* On the other hand, 42% (n=40) of HCPs did not agree that HMs were safe. Some participants stated that HMs may be more dangerous than CWM due to the lack of evidence to support their use. Various reasons participants felt

HMs were not safe included the lack of clinical evidence to support their use, the lack of regulation of HMs, and problems of quality and contamination. Several participants commented on the need for proper regulation to be able to determine the safety of HMs. Participant 36 (a consultant physician) said, *"Whilst only a small amount of harms are caused - I cannot call them safe because in some instances they are not. I think that all in all more regulation is needed and then I would consider the patient's views in taking a cautious approach to use of herbal remedies in some conditions,"* and participant 44 (a pharmacist) who noted, *"Traditional herbal medicines need to be regulated better, I have seen recent reports of hazardous contaminants."*

An interesting statement was made by participant 15 (an anaesthesia registrar) who claimed, *"It's more complex than whether they're safe or not - it depends on the dose, what other medication the patient is on & the interaction between them (if any). Often, herbal products have inconsistent concentrations between batches & therefore the true dose is never known. There have been instances of additives not listed on packaging also being included in the product - I feel generally these products are an unknown quantity because the contents, concentration & production methods are subject to much lower standards of scrutiny than tablets. They are drugs just as much as tablets are, the 'herbal' label lulls people into thinking there are no possible side effects - this is not true, nor is it safe."* This reiterates that there are numerous factors which can affect the safety of HMs including: the dose (high doses may cause toxicity), co-morbidities (taking HMs while taking CWM increases the risk of interactions), time of use (before surgery could impair blood coagulation or cause complications, during pregnancy or lactation could affect the baby), adulteration (the wrong species selected), or contamination (heavy metals or other active constituents) (Upton and Romm, 2012). Furthermore, participant 59 stated, *"For most conditions, herbal medicine is unnecessary, ineffective and the risk of interactions with pharmaceutical drugs too high to recommend using them for any condition. Contamination (accidental, natural and deliberate) means their safety will always be in question."*

Some herbal products are now prohibited or have restricted use in the UK due to the safety concerns associated with them. The MHRA document '*Banned and restricted herbal ingredients*' updated in December 2014, lists all the products which are subject to restrictions in the UK. It includes completely restricted products such as aristolochia, Kava-kava, and aconite (*Aconitum* species) traditionally used for poison arrows. Other products which can only be prescribed by a registered doctor or dentist include: foxglove (*Digitalis purpurea* L.) and opium (*Papaver somniferum* L.). Some products can only be purchased from a registered pharmacy under the supervision of a pharmacist such as, Mistletoe berry (*Viscum album* L.) and Belladonna (*Atropa belladonna* L.) (MHRA, 2014d). Before

concerns of the risks, adverse effects, and fatalities were recognised some of these products were widely available; but, over time as more reports documented the risks associated with them, they were withdrawn from the market.

All drugs which have a marketing authorisation have to undergo extensive research to validate the risks and benefits of treatment; whereas, HMs may be consumed without any clinical research to support their use. The fact that HMs have been used for hundreds of years does not suggest they are safe (Barnes et al., 1998). Herbal products known to cause adverse effects or which may be harmful can be avoided; however, this information may not always be known and the long term implications remain to be uncovered. The risks of herb-drug interactions can affect the efficacy of conventional medicines which may threaten the pharmaceutical care of patients (Pharmaceutical Press, 2013). Participants in this survey also made reference to the fact that HMs were harmful as their patients were more reluctant to take CWM if they were taking HMs, making health problems worse and more difficult to manage once they had exacerbated. There were several concerns raised over the safety of HMs; nevertheless, with better information sharing between patients and their HCPs, reporting of ADRs, and more research on HMs the safety profiles for herbal products can be better understood.

Question 12: ***Would you ever recommend herbal medicines to your patients?***

Various reasons for not recommending HMs to patients were identified by 65% (n=62) of participants, including: participant 51 (an independent public health specialist) who said he would not recommend HMs, "*Because of the antipathy and overabundance of perceived problems related to herbal remedies by the medical profession,"* participant 56 (a dietician) who claimed there was, *"Not enough evidence to support the use of herbal medicines and potential interactions with Western medicines,"* and participant 69 (a doctor) who stated, *"It all depends on the evidence base! Great damage is done when people take alternative medicine instead of medicine proven to work."* The Mintel Report (2009) claims that doctors and pharmacists may be uncomfortable referring patients to herbalism due to the lack of regulation, this was also observed within this research sample whereby HCPs said that the lack of regulation was one of the main reasons for not recommending HMs. This is further supported by the findings of the DTB survey of HMs which found that 84% (n=138) of HCPs surveyed did not think HMs were well regulated (DTB, 2010).

Some of the other reasons participants in this study refused to recommend HMs to patients included:

Participant 16 (clinical pharmacist), *"Difficult to quantify, as each case would vary in view of co-morbidities and individuals."*

Participant 22 (medicines information pharmacist), *"As a healthcare professional recommendations on treatment should be evidence based...evidence for efficacy and safety of herbal remedies is lacking, therefore I would not recommend herbal remedies."*

Participant 32 (senior pharmacist), *"There are not enough large comparable clinical trials into herbal medicines to warrant advising patients to use them instead of Western medicines. There is insufficient data for mixing herbal medicines and Western medicines, much of the advice provided to my patients if they state they want to use herbs as well as Western medicine is based on limited data and theory."*

Participant 77 (GP), *"I work on evidence based medicine principles and therefore unless trial evidence available I could not in good faith recommend them."*

Participant 84 (pharmacist), *"I do not recommend herbal remedies due to concerns over interactions with conventional medication. I think they could play a role in prevention/treatment of health conditions but there is variable quality between preparations which again means I do not feel I can recommend them. I do not see the harm in someone not taking a conventional medication trying herbal remedies."*

According to the GPhC standards of conduct, ethics, and performance, pharmacist are not allowed to recommend any remedy where they have any reason to doubt the safety or quality (Rees et al., 2014). In addition, pharmacists should only offer advice on HMs if they have had suitable training or knowledge. Some participants who said they would not recommend HMs went on to say they would recommend HMs if they had better knowledge of HMs; like participant 11 (a pharmacist) who claimed, *"I would recommend herbal remedies to patients if my knowledge on it was sufficient. As I do not know much about it and how it works to treat different conditions, I cannot really recommend it."* Similarly to the findings of this research, Koh et al. (2003) found that many pharmacists like doctors did not believe they had the basic knowledge about HMs to be able to recommend them safely.

Other participants who said they would recommend HMs if there was more evidence to support their use such as participant 69 (a doctor) who said, *"Herbal remedies are fine, but I will only prescribe them when I have evidence that they work better than conventional therapy and that they are safe to use within the context of the pharmaceutical regimens the*

patient is already on." Furthermore, participant 106 (a pharmacist) claimed, "*The biggest problem with traditional herbal remedies is lack of information. The most common query I get regarding herbal products is "Is this safe with my other medication?" While there are very good books around (e.g. Stockley's Herbal Medicines Interactions) and advice (e.g. from the NPA) the books are rarely available in the average pharmacy and a phone enquiry cannot always give the immediate response that a customer may want. As a result it is frequently necessary to err on the side of caution and say that I cannot recommend it as I do not have sufficient information on the product."*

Just 35% (n=34) of participants said they would recommend HMs to their patients. The types of herbal remedies participants said they would recommend are summarised in Table 3-7. The most common herbal remedies participants said they would recommend included turmeric for various ailments such as cough, sore throat, skin conditions, and wound healing. Honey and lemon for coughs and colds, and evening primrose oil for low mood. Some of the reasons participants said they would recommend HMs to their patients included:

Participant 12 (pharmacist), *"The use of herbal medicines is safe if used in context of other co-administered drugs and with full knowledge of the effects of the herb."*

Participant 20 (clinical pharmacist/ senior lecturer), *"Alongside allopathic medicines provided the patient consults the pharmacist - I think the patients' faith in day to day traditional herbs and spices also helps them psychologically."*

Participant 27 (optometrist student), *"Because it will most probably be preservative free meaning less adverse reactions from patients. Most likely to have less side effects. More positive effects then negative even if used long term. Herbal medicines are sustainable."*

Participant 70 (pharmacist/ researcher), *"If they are effective and safe, why not?"*

Participant 88 (pharmacist), *"Because it is safer, effective and with less side effect than conventional medicine."*

Table 3-7 Herbal Remedies Healthcare Professionals Would Recommend To Their Patients

Participant Number:	Herbal Remedies Healthcare Professionals Would Recommend:
2	*Turmeric for digestive complaints.* *Rhodiola for energy.* *Saw Palmetto for Libido.*
3	*Using ginger in tea or in foods to help with sickness.* *Green tea to help with digestion and due to anti- oxidant properties.*
6	*Tea bags for dry eyes.*
18	*Peppermint products for IBS.*
21	*Turmeric as it works for me!*
23	*For minor ailments such as cough and cold: honey and liquorice, honey and lemon.* *For Indigestion: fennel seeds, mint, aniseed.*
26	*Aloe vera creams, neem soap-is effective for patients who have skin conditions such as eczema.*
46	*Very simple remedies which I have tried and tested, mainly those involving turmeric and honey as previously stated.*
47	*For examples garlic against infections.*
49	*Yam for arthritis.*
63	*Passion flower for stress.* *Agnus castus for PMS/ cycle regulation.* *Saw palmetto for prostate (BP) related lower urinary tract symptoms.*
64	*For mood - St. John's wort or evening primrose oil.*
68	*Black cohosh.* *Evening primrose oil* *Saw palmetto.*
73	*Of course most elements and biological substances have active ingredients and while all are bad for you in excess- even water - many have been synthesised, including the first manufactured drug aspirin (from salicylic acid, originally from tree bark). In fact when herbal remedies are proved in scientific trials to have beneficial effect they are absorbed in mainstream medicine. What is left is usually of minor or no value, based on placebo effect and encouraging pseudoscience.*
74	*Honey and lemon is much better than cough syrups.*
81	*Peppermint for digestive issues.*
82	*Echinacea proven remedy anything that I have the evidence for.*
91	*Typically, for constipation- figs, prunes and pears. Natural constipation relief with the added benefit of all other associated nutrients.*
103	*Prevention as part of balanced diet.*
108	*I've recommended using cider vinegar to help with arthritis as I've seen that it helped my mum.*

Howard et al. (2001) found that in their survey of 70 pharmacists, 53% claimed to use dietary supplements themselves, they later went on to conclude that personal use correlated to an increased likelihood of making a recommendation to a patient. This can also be seen from the results of this investigation; participants who used HMs were generally more inclined to recommend them to their patients (Figure 3-8). Newall et al. (1996) say that HCPs need to be aware of the suitability of herbal remedies, even if HCPs do not use HMs themselves or believe they are effective they should still have some awareness about HMs as their patients may be using them.

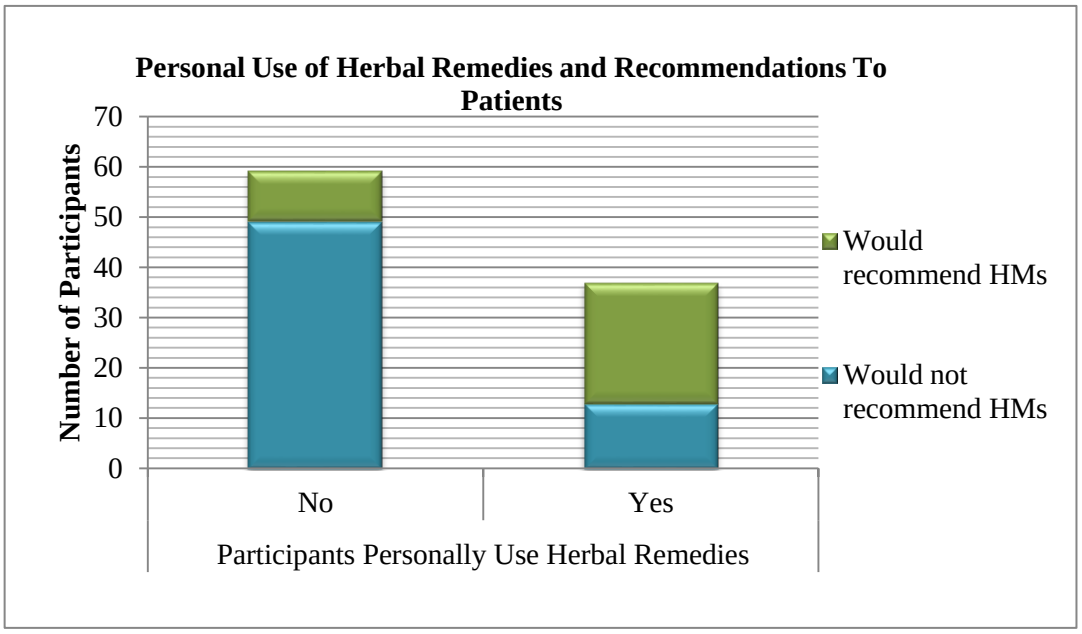


Figure 3-8 Correlation of Healthcare Professionals' Personal Use of Herbal Remedies to Recommending Herbal Remedies to Patients

Heuschkel et al. (2002) found that one of the reasons respondents in their survey, about the use of CAM in patients with IBD, said they would use complementary therapies was if they were recommended by a doctor. The results from this survey suggest that some HCPs would recommend HMs, while others may have their reservations. Generally, the reasons for not recommending HMs were due to concerns of potential interactions with CWM, reduced compliance with prescribed medication, lack of evidence to support the use of HMs (i.e. limited safety and efficacy data), and HCPs personal lack of knowledge of HMs. Participants who were happy to recommend HMs had positive attitudes towards HMs, and often had tried HMs themselves. If there was better evidence to support the use of HMs and HCPs were more competent many said they would recommend HMs to their patients.

Question 13: ***Did you receive any information about herbal medicines during your professional training?***

As the literature available on how much information is passed on to undergraduate HCPs was limited this question was designed to try and establish whether or not HCPs in the UK had received any training on HMs. Over a third of participants (35%, n= 34) said they did receive some information on HMs while undertaking their professional training. This was exemplified by participant 33 (a senior lecturer in law, ethics and pharmacy practice) who said, *"When I trained (in the 1970's) there was a large amount of pharmacognosy taught and that did cover materials of plant origin but not herbal remedies specifically."*

However, a large number of participants (65%, n=62) said they did not receive any information about HMs during their training. HCPs are at the frontline of patient care so need to be competent in giving advice and treating patients appropriately especially if they may be taking HMs. If HCPs are not adequately trained it could affect patient care, increase the risk of interactions with CWM, and adverse effects may go undetected.

Question 14: ***Do you think enough training is provided to you about herbal medicines?***

The results from this question revealed that the vast majority of participants (74%, n=71) said they did not think enough information was provided to them on HMs. Participant 67 (a professor emeritus of surgery and medical humanities) stated, *"Our students should be taught that there are no metaphysical difference in biological activity between herbal and pharmaceuticals."* Koh et al. (2003) conducted over 400 surveys on pharmacists in Singapore to find out about their use, knowledge and attitudes towards CAM; they found that 90.5% (n=380) felt not enough information on CAM was provided during their training.

Although regulatory bodies such as the GMC and GPhC do require undergraduate students to be informed about CAM, they do not specify the content. As the brief is often so broad (i.e. the GPhC standards for education states *'complementary therapies'*) this leaves schools open to decide what they think is suitable to meet the requirements (GPhC, 2011). Research by Smith (2011) found that in some medical schools, in the UK, a single lecture was deemed sufficient. It may be useful for regulatory bodies to clarify the extent to which CAM should be taught to students (Smith, 2011) to better prepare future HCPs to deal with patients taking HMs.

Some participants in this study (26%, n=25) said they thought enough training was provided; for example, participant 85 (a pharmacist) who stated, *"I know about herbal medicines as I studied pharmacognosy during my bachelor's degree in India";* however, this participant did her pharmacy degree in India where training on HMs is commonly included in the curriculum. Furthermore, Participant 61 (a GP) who said he did not receive any information on THM while training, nor did he think enough training was provided then went on to say, *"It's difficult to see how further training in a totally different method of medicine, using preparations with significantly varying concentrations of active ingredients and unregulated or tested by MHRA could be fitted in to an already overloaded curriculum."* The comments made by participant 61 suggest that the intensive training programmes HCPs endure are already straining; similar responses were also seen by Smith (2011). When Smith (2011) contacted UK medical schools to investigate the extent of inclusion of CAM education in undergraduate courses it was revealed that schools felt there was a lack of interest from staff and students in CAM, limited funding, and most importantly the programmes were already congested. Newall et al. (1996) claims that, *"a pharmacist should be able to advise the consumer on the rational and safe use of all medicines; thus, a pharmacist should be fully informed about the quality, safety, and efficacy of herbal medicines."* However, if this vital information is not transmitted to HCPs while they are training it would be difficult for them to gain this knowledge. Research conducted by the DTB (2010) explored how well informed HCPs felt that doctors were about HMs; 75.5% felt doctors were '*poorly informed*' about HMs, while only 21.5% felt that doctors were '*well informed*'.

As the popularity of HMs increases, more high quality research to support the use of HMs is required. Vickers (2000) claims that research on CAM is being conducted worldwide reflecting the growing awareness of the use of HMs. This will enable HCPs to be educated about HMs with reliable evidence based guidelines, an issue which was raised by many participants in this investigation. Regulatory bodies should make requirements for CAM education clearer so HCPs are equipped with the knowledge to deal with HMs queries competently. Educating future HCPs and providing continual professional development (CPD) programmes to encompass HMs will help HCPs make better informed decisions about HMs.

Question 15: ***Any other comments:***

The final question gave participants the opportunity to share any comments and feedback. Table 3-8 summarises the remarks made by participants. Most of the comments were generally about the structure and content of the survey. Some participants, such as participant 22, were very engaged with the questionnaire and had several points they wanted to highlight. There were concerns that just participants who used or had knowledge about HMs would complete the survey giving biased results; however, the comments suggest participants with a range of opinions completed the survey, not just people who are in favour of HMs.

Table 3-8 General Comments from Healthcare Professionals

Participant Number:	General Comments:
2	*Very nice survey, number 10 - it depends what health conditions....*
12	*In line with question 12, more information is needed rather than an outright rejection by healthcare practitioners.*
16	*All the best with the project!*
22	*Your survey does not give the opportunity to say that I am happy for patient's to take alternative remedies as long as they are safe, of good quality and do not interact with other drugs. I am aware of the beneficial effects (may be placebo) of the patient being empowered by being in control of their own therapy. Taking alternative remedies should not stop a patient reporting the condition/side effect that they are treating to their clinician. Very little information is available on South Asian alternative remedies. Have great concerns about quality of products available.*
30	*Use of herbal medicines is common amongst people living with HIV. This might be an area to investigate in the future.*
43	*Difficult to answer some questions which are closed questions and therefore black and white.*
49	*Would love to hear more.*
50	*Lot of potential. Needs research.*
64	*I am in mental health and do not prescribe so limited experience of patients' prescription medicines for things other than depression and pain.*
66	*What matters is not what people think, but what the evidence says. So there is no yes or no answer for question 10 and 11.*
81	*Would have been nice to have a list of herbal medicines plants spices to help channel thoughts with regards to answers in survey.*
90	*It would be nice if various traditional medicines could be included in Western medicine however it is very difficult and not practical.*

3.7 Healthcare Professionals Survey Summary

The HCPs survey was published online using the SurveyMonkey software, and distributed via social media (Facebook and Twitter), which enabled a range of HCPs from across the UK to participate. In total 112 surveys were attempted with participants dropping out at various stages; although 93 surveys were completed, responses for incomplete surveys were also used in the analysis to avoid losing some valuable data. This research has provided a unique insight into HCPs personal and professional opinions of HMs. As there is limited research into UK based HCPs views of HMs the results from this study can be used to contribute to this discipline.

The use of social media to conduct scientific research has only gained attention in the last few years (Sarker et al., 2015), and proved to be highly valuable while conducting this research. By using social media to distribute the link to this survey it enabled HCPs from across the UK to be contacted to participate in this research. A range of different HCPs comprised this research population, instead of being limited to a single group of HCPs which would have been the case if postal questionnaires were used. As the survey was published online via SurveyMonkey it was easy to create, modify, and track the progress of the questionnaire. Other benefits of conducting this research using social media included the fast and efficient distribution of the survey, lots of responses were collected in a short period of time, and it was cheaper than printing and posting the questionnaires. The disadvantages of using social media while conducting this research included the lack of control of who was sharing the link, who was completing the questionnaire, and participants dropping out partway through. If the surveys were conducted face to face the process could have been regulated better with fewer participants dropping out or missing questions (Moore, 2000). The main issue was the lack of control over who was completing the questionnaire. One completed survey raised concerns about whether or not the participant was a HCP from the UK. In the introductory statement before the questionnaire there was great emphasis on 'UK based HCPs'. When the link for the survey was posted on Twitter and Facebook it was also emphasised: "*If you are a UK based healthcare professional please take a few minutes to complete this survey."* For this reason later a question was added at the end of the survey to determine whether or not participants did or had worked in the UK, to try and overcome the issue of HCPs from outside of the UK being included in the analysis. Finally, one of the most time consuming parts of this investigation was the ethical approval process, as the use of social media and online surveys is a relatively new way of conducting research there were no guidelines for ethical approval (Beninger et al., 2014). Uncertainty amongst internal (DMU) and external (NHS) ethics committees meant the ethical review process was lengthy and complicated. Despite some of the drawbacks encountered during this study, the results

suggest it has been a very useful way of conducting research. HCPs from across the UK, the researcher would not have ordinarily come into contact with were able to take part. Responses from a variety of different HCPs representing various professions, age groups, and ethnicities were able to contribute their knowledge and opinions. As the survey was available online it enabled participants the freedom to complete it at their own leisure and pass it on to other HCPs they knew.

The HCPs that formed this survey population were from a range of professions including pharmacy, medicine, dentistry, optometry, research and academia. Participants from various ethnic groups completed the survey, making it possible to compare ethnic differences between knowledge and use of HMs. Just under half of the participants (45%, n=50) said the use of HMs was part of their traditional family and cultural background. There was a statistically significant association between the traditional use of HMs and ethnicity. SA ethnic groups were more likely to say HMs were part of their family and cultural background than any of the other ethnic groups; while White British/ Irish participants were more likely to claim that HMs were not part of their traditional heritage. Over a third of participants (38%, n=42) said they used HMs themselves, these participants documented a large assortment of HMs they knew of or used. The most common products recalled by participants included turmeric, ginger, mint, honey, lemon, and Echinacea. Participants (53%, n=54) mentioned a wide range of HMs they knew of, for the four conditions listed: arthritis, cough, diabetes, and indigestion. In comparison to the findings from the SATMED questionnaire a more diverse range of herbal products were known and documented by the HCPs in this survey; products native to Asia (e.g. ajwain and tulsi) and Western countries (e.g. camomile and Echinacea) were mentioned. The diversity of products mentioned by HCPs could be because the questionnaire was open to all ethnic groups, and not restricted to just SA participants like the SATMED questionnaire. The results from this analysis revealed that HCPs who completed this survey had variable knowledge of HMs; some participants had extensive insight into a broad range of HMs, while others had limited knowledge. A difference in knowledge of HMs between ethnic groups was also identified, as more SA HCPs recalled HMs in comparison to White British/Irish HCPs. This knowledge difference could be linked to the traditional heritage of HMs amongst SA cultures which SA participants referred to in both this survey and the SATMED questionnaire. From the comments of several participants it was evident they were keen to learn more about HMs if there was evidence to support the claims associated with the use of HMs.

When HCPs in this study were asked if their patients told them about their use of HMs 65% (n=64) said they did. However, when participants from the SATMED questionnaire were asked whether or not they told their doctor or pharmacist about their use of HMs, 69%

(n=73) said they did not tell their doctor while 82% (n=87) did not tell their pharmacist. This is supported by the findings of several studies which have found patients do not always tell their HCPs about their use of HMs. Giveon et al. (2004) found that 44.7% of participants did not tell their doctor about their use of HMs, whilst 11% did but only rarely. Results from the Ipsos MORI report (2008) on public perceptions of HMs found that 56% of participants felt it was important to tell their doctor about their use of HMs; interestingly they found that Asian and Black minority ethnic groups were less likely to disclose information about their use of HMs to their doctors (Ipsos MORI, 2008). The qualitative results from the SATMED questionnaire revealed that SA participants did not want to tell their HCPs about their use of HMs as they were afraid of being treated differently or not treated at all. The high rates of under reporting the use of HMs by patients may be influenced by several factors, of which ethnicity may be one (Williamson et al., 2013; Ipsos MORI, 2008); this could explain why the results for not reporting the use of HMs to HCPs was so high in the SATMED survey. The HCPs in this survey revealed that their patients informed them about their use of HMs fairly frequently. The importance of accepting and understanding cultural differences is vital for HCPs to be able to build rapport with their patients; as Staines (2011) highlighted, that negative attitudes of HCPs towards alternative therapies could be detrimental to the relationship with their patients. Building trust will help patients feel at ease when disclosing information about their use of HMs to their HCPs; this is vital for providing appropriate care for patients, identifying adverse drug reactions (ADRs) and interactions with HMs.

Numerous herbal ADRs were noted by 31% (n=30) of participants. Some ADRs identified were considered to be minor effects (e.g. local irritation caused by using tea tree oil); whereas, others were more fatal and sometimes life threatening (e.g. hypoglycaemia caused by karela, or deranged liver functions caused by Kava kava). As people using HMs often 'self-prescribe' the risks associated with ADRs may not be known; Barnes et al. (1998) found that those who experienced ADRs (minor or serious) from HMs were less likely to see their GP than if it was an ADR caused by CWM. Although efforts to improve herbal pharmacovigilance have been implemented (i.e. the yellow card scheme has been extended to include herbal ADRs), herbal ADRs are still underreported and go unmonitored (Barnes, 2003). This reinforces the importance of HCPs being competent in recognising and reporting incidents associated with HMs.

The most common herb-drug interaction reported by 33 participants in this research involved SJW. SJW was the third best-selling herbal remedy in the UK in 2013 (Ministry of Foreign Affairs, 2014). Its traditional use for mild depression and anxiety has become so popular its status as a traditional herbal remedy has been questioned. It is one of the few HMs for which there is extensive research and clinical evidence to support the traditional health claims

associated. It could be due to the wealth of literature available for SJW that makes it so well known to HCPs. The most common CWM which participants identified as being affected by HMs was warfarin. Many participants described HMs which could potentially affect the efficacy of warfarin including garlic, gingko, SJW, and cranberry juice. Polypharmacy with CWM is common practice in patients with multiple health conditions; combining HMs into the mixture could increase the risk of ADRs and interactions (Edwards et al., 2012). Due to the unknown risk of herb-drug interactions some HCPs believe it is best to avoid taking HMs alongside CWM (Fugh-Berman and Ernst, 2001). Almost two thirds of participants (63%, n=62) in this survey were aware of herb-drug interactions and documented some of their knowledge. The 37% (n=36) of participants who said they were not aware of interactions between HMs and CWM highlighted the importance of educating HCPs about the potential risks associated with consuming HMs alongside CWM.

Despite the range of ADRs and herb-drug interactions documented by the research participants their views on the safety and efficacy of HMs were mixed; 66% (n=63) of participants thought HMs were effective for treating and preventing various health conditions, and 58%, (n=56) said they were safe. Some participants felt the long standing use of HMs throughout history was sufficient evidence to support the use of HMs, while others made reference to the lack of evidence based medicine and scientific validation of HMs. The 42% (n=40) of participants who said they did not think HMs were safe and the 34% (n=33) who did not think they were effective felt the lack of clinical evidence for HMs meant they could not make an informed decision to determine the safety and efficacy of HMs. In addition, without randomised clinical trials to prove the efficacy of HMs many HCPs felt reluctant to recommend HMs to patients. Nevertheless, it was observed that those HCPs who had personally tried or used HMs were more likely to recommend them to their patients. Vickers (2000) suggested that recent advances in research have provided some good evidence to support the use of some complementary therapies; in addition, more research is being conducted around the world to provide evidence to support the use of CAM. Funding to support research has also been increased, although it still lags behind other areas of research and is still fairly restricted (Vicker, 2000; Cant et al., 2012).

The significant majority of participants (65%, n=62) claimed they were not given any information on HMs during their professional training; while, even more participants (74%, n=71) believed that not enough training was provided on HMs. These results have given an insight into what HCPs in the UK think about the current level of education on HMs provided to them. The analysis reveals that HCPs are not satisfied with the existing level of training they receive on HMs, and many felt incompetent giving advice about HMs to their patients. Although regulatory bodies such as the GMC and GPhC do set standards which

require schools to include some information about CAM there is no defined syllabus; and in some cases a single lecture was deemed sufficient to meet the standards set by regulatory bodies (Smith, 2011). Very little high quality research on CAM exists (House of Lords, 2000), this could be a key reason as to why it is not extensively taught in undergraduate courses. More money is being invested in exploring the risks and benefits of CAM, and high quality research is being conducted worldwide (Vicker, 2000); this will provide better resources to educated HCPs so they can make informed decisions about HMs. Several participants expressed their interest in learning about HMs, especially if there was scientific evidence to support their use.

There are some very useful texts to help HCPs learn about HMs, such as Stockley's Herbal Medicines Interactions (Williamson et al., 2013) and Phytopharmacy – An Evidence Based Guide To Herbal Medicinal Products (Edwards et al., 2015); however they are not readily available to all HCPs. The British National Formulary (BNF) is a vital resource for HCPs and is available to all doctors and pharmacists and many other HCPs. The BNF July 2015 edition explains why information on HMs is not included in the BNF; it states, *"while it would be useful to have information about herbal medicines in the BNF, in order to provide reliable and authoritative information on homeopathic or herbal medicines, it would be necessary to set up a parallel structure with specific expertise in complementary medicine. It would be very difficult to achieve this and to include all the information in the BNF."* Having access to texts which would help HCPs make informed decisions about the safety and efficacy of HMs will help improve HCPs knowledge of HMs, relationships with patients, and increase HCPs confidence in advising patients about the use of HMs (Pokladnikova and Lie, 2008).

As the popularity of HMs continues to grow (Zhang et al., 2012), and HMs become more integrated into Western healthcare (Vickers, 2000), the importance of HCPs being aware of the risks and benefits of consuming HMs alone or in conjunction with CWM will become more important. In order to ensure patients are receiving the correct pharmacological care it is vital for HCPs to deduce their patients' use of HMs; a concept which needs to be reinforced to HCPs in the UK. Changes to the regulation of HMs and more clinical research are providing evidence based support for the safety and efficacy of HMs. Furthermore, better training and education on HMs for future HCPs means they will be equipped with the knowledge to identify potential ADRs and interactions, and advise patients on the safe use of HMs. There are very few studies which have explored UK based HCPs views on CAM (Smith, 2011), and little research actually focuses on just HMs. The results from this investigation have enabled an insight into HCPs personal use and professional views of HMs to be uncovered; providing valuable data for future research.

4.0 Chapter 4 – The Tulsi Project

4.1 Introduction

The Tulsi Project was developed after considering the results from the main South Asian Traditional Medicines (SATMED) questionnaire (Chapter 2); which explored the current use of traditional herbal medicines (THM) by South Asian (SA) diasporic communities, in the United Kingdom (UK). DNA authentication projects conducted by colleagues at De Montfort University (Chapter 2, section 2.6.2, p37) and other researchers have highlighted how DNA barcoding has been used to identify adulterant and substitute species in herbal medicines (Howard et al., 2009; Kumar, 2015; Seethapathy, 2015) have inspired this research.

Tulsi (Holy basil, *O. tenuiflorum* L.) was identified as one of the most popular herbal ingredients used and known by the SATMED participants. This plant stood out from all of the other common herbal products identified such as ginger (*Zingiber Officinale* Roscoe) and turmeric (*Curcuma longa* L.) for several reasons. Firstly, the numerous health benefits associated with tulsi ranged from minor skin ailments to long term health problems like asthma and diabetes (Chapter 2, Section 2.6.2, p37). When participants were asked if they grew any plants for medicinal purposes, tulsi was one of the main plants, after mint, which was grown; tulsi seeds were commonly brought to the UK from Africa and India. In addition, when participants revealed what products they imported to the UK from abroad it was commonly dried tulsi leaves or seeds; with reference to the poor growth conditions and low survival rates of the plant in the UK. The religious significance of tulsi was also noted as participants referred to the value of the plant for Hindus.

After considering all the information collected, it was evident that participants grew their own tulsi plants, imported material from abroad (Africa and India), and bought commercial products from suppliers in the UK, online, and abroad. A variety of different tulsi plants were recognised by participants, with frequent references made to Raam, Shyam (Krishna), and Vana tulsi. Raam and Shyam tulsi are varieties of *O. tenuiflorum* which is native to Asia; whilst some authors claim Vana tulsi is *O. gratissimum* L. (Joshi et al., 2012). As participants revealed that they grew plants from seeds they acquired from abroad, an interest in the species of tulsi plants grown in the UK developed; the applications of DNA analysis for species authentication to identify tulsi species were then explored.

DNA barcoding is a relatively new technique that can be used to discriminate between different plant species, and identify any contaminants or adulterants. The literature around this topic is ever expanding and it is anticipated that the results from this investigation will

have a novel contribution. A range of tulsi samples were collected which were subjected to DNA analysis to determine their species. Samples were collected from research participants (community samples), as well as commercial samples, and authenticated species for the identification process. This research involved a multi-disciplinary approach by combining social science research methods with molecular techniques.

4.2 Aim of the Tulsi Project

The aim is to investigate the cultural and commercial value of tulsi, *Ocimum tenuiflorum,* amongst diasporic South Asian communities in the UK; and to authenticate tulsi samples using DNA-based analytical techniques.

4.3 Objectives of the Tulsi Project

1. Explore the cultural and commercial values associated with tulsi, in South Asian communities in the UK.
2. Collect a range of tulsi products and perform DNA analyses in order to: classify species, identify genetic variations, and isolate adulterant species.

4.4 Background to the Research

This section will give an insight into the origins, cultural values, medicinal uses, and botanical identity of tulsi; in order to establish the importance of this research. An explanation of the traditional and new research methods used for medicinal plant identification will highlight the potential applications of DNA barcoding.

4.4.1 Origins of Tulsi

Tulsi is native to tropical Asia, it grows wild in warm regions. It has been used in South Asia, China, the Middle East, Africa and Australia for centuries (Maimes, 2004). With the migration of people, the plant has become available around the world, and varieties are even grown in the UK. As the tulsi plant travelled West, it became known to Christians as "holy" basil as is reflected in its Latin binomial, *O. sanctum* L. (now referred to as *O. tenuiflorum* L.). The scared plant was given as a gift in the annual celebration of the birth of St. Basil, as people gave it the status as a ‘gift from Christ’ *(Miller and Miller, 2003).*

Tulsi is used in various traditional medical systems including: Ayurveda, Siddha, and Unani. Ayurvedic scriptures refer to tulsi as one of the main pillars of herbal medicine, first mentioned in the Rig Veda around 1500 BC. It is widely incorporated in traditional Indian cuisine, cosmetics, herbal remedies and religious ceremonies (*poojas*). The whole plant can be used, seeds, roots, leaves, flowers and even the stem (Prakash and Gupta, 2005). The woody stem is often used to make tulsi beads, worn as necklaces or bracelets believed to provide physical protection, and even made into rosaries for spiritual protection (Miller and Miller, 2003). Daily use of tulsi is believed to help maintain balance of the *chakras,* energy centres of the body (Winston and Maimes, 2007).

Tulsi is one of the most sacred plants on the Indian subcontinent, as it represents a Hindu goddess, Virinda Tulsi. Legend has it that Virinda was married to a demon called Jalandhar who was destroying the world. Due to Virinda's purity and devotion to God her evil husband was immortal. The only way to kill Jalandhar was if his wife's purity was tarnished. Lord Krishna disguised himself as Virinda's husband and tainted Virinda's purity; which led to the death of Jalandhar. Virinda felt she had been betrayed by the Gods and threw herself onto her husband's burning pyre, to relieve herself from the shame of cheating on her husband. From this it is said her soul was transformed into the tulsi plant and Lord Krishna vowed to marry her to take away the shame. Hence, every year the wedding anniversary, *Tulsi Vivah,* is celebrated by Hindus around the world (Figure 4-1). It is Virinda Tulsi's purity and devotion to God which makes Hindus believe the plant is so pure and sacred; thus the plant is incorporated in religious ceremonies, used as religious offerings (*Prasad*), and added to the burning pyre of the deceased where it is believed to aid the passage to heaven (Shatri Ramnik Dev, 2013). Tulsi plants are supposedly kept in every Hindu temple and most households where it is thought to be a blessing if the plant grows (Wagner, 2010).

Figure 4-1 Tulsi Wedding at Shree Laxmi Narayan Temple, Birmingham

4.4.2 Medicinal Benefits of Tulsi

The '*elixir of life*,' tulsi is known as a *rasayana*, a herb with life prolonging and rejuvenating properties, which helps prevent and cure diseases (Miller and Miller, 2003). Tulsi is considered one of the most powerful herbs as it is used for treating a vast array of medical disorders; including conditions which affect the skin, the cardiovascular, endocrine, respiratory, and central nervous system (Winston and Maimes, 2007). Traditionally tulsi has been used to treat asthma, bronchitis, diabetes, diarrhoea, eye disorders (chronic conjunctivitis, cataracts, and glaucoma), fever, insect bites, snake bites, malaria, and a variety of skin diseases. Tulsi is also known to have antibacterial, antifungal, antiviral, antidiabetic, anticancer, antifertility, anti-inflammatory, adaptogenic, analgesic, cardioprotective, and hepatoprotective properties (Prakash and Gupta, 2005; Philip and Unni, 2011; Singh et al., 2012). Different parts of the tulsi plant including the seeds, roots, stems, and leaves are known to possess therapeutic properties and are used to formulate herbal remedies (Prakash and Gupta, 2005). There are various ways to consume tulsi in order to reap the medicinal benefits. Fresh or dried tulsi leaves can be eaten, used in cooking, infused in tea, hot water, or bath water. A variety of commercial tulsi products are also available including: capsules, creams, juices, oils, shampoos, soaps, tablets, teas, and tonics.

Human and animal studies have suggested tulsi can be useful for diabetes as it has been found to alter glucose regulation via several pathways (Chattopadhyay, 1993; Vats et al., 2002; Prakash and Gupta, 2005). Agrawal et al. (1996) administered 2.5g of tulsi leaves to type 2 diabetic patients for sixty days and observed a decrease in fasting and post-prandial blood glucose. Tulsi has been shown to reduce blood glucose levels, and increase glycogen stores and insulin levels in the body (Joshi et al., 2012). Traditionally tulsi is believed to have been used as a contraceptive and to improve libido. The literature available on the antifertility and abortive effects of tulsi are dated (Khanna et al., 1986), but precaution in pregnant women are still taken (Prakash and Gupta, 2005). Studies in female rats found a reduction in implantation, full term pregnancies and a reduced litter size after the administration of tulsi (Vohra et al., 1969; Khanna et al., 1986). Animal studies suggest tulsi can temporarily reduce sperm count and sperm motility (Ahmed et al., 2002), and increase libido (Pande and Pathak, 2009). Hence, in India tulsi has traditionally been used as a male contraceptive (Sethi et al., 2010). Research even documents the powerful antibacterial effects of tulsi in treating the sexually transmitted disease, gonorrhoea, caused by *Neisseria gonorrhoea* which is sensitive to extracts of *O. tenuiflorum*; Shokeen et al. (2005) refer to it as being as good as penicillin and ciprofloxacin.

4.4.3 Active Compounds in Tulsi

The chemical composition of tulsi is complex and can vary between different cultivars (Rahman et al., 2011). The quantities of chemicals found in plants can be affected by the growing conditions, stage of harvesting (young or mature plant), processing and manufacturing stages, and storage conditions (Pattanayak et al., 2010). Tulsi plants contain a variety of saponins, flavonoids, triterpenoids, tannins and essentials oils; including, eugenol, methyl eugenol, eugenic acid, urosolic acid, carvacrol, linalool, limatrol, caryophyllene, and methy chavicol (Pattanayak et al., 2010; Rahman et al., 2011).

Tulsi leaves contain the phenolic compounds eugenol and methyl eugenol which form part of the plants' essential oil content. Eugenol is responsible for the spicy aromatic fragrance produced by the plant, as well as some of the associated health benefits. Eugenol has antiseptic, anaesthetic, antibacterial, antifungal, and antioxidant effects. Quantitative variations have been seen in the composition of essential oils in tulsi plants grown in different parts of India, different seasons of harvesting and growing conditions (Prakash and Gupta, 2005).

4.4.4 Precautions with Tulsi

Traditionally tulsi is avoided in pregnant women as it has been known to cause miscarriage and was used to induce abortion. Ursolic acid, one of the major constituents found in the leaves, is believed to have antifertility effects (Prakash and Gupta, 2005). Batta and Santhakumari (1970) demonstrated the antifertility affects in mice by administering a dose of 200mg/kg of tulsi in a benzene vehicle to pregnant mice. Animal studies have shown the antifertility effects but the results remain dated and inconclusive (Gardner and McGuffin, 2013). Tulsi was traditionally considered to be a male contraceptive (Sethi et al., 2010) and has been associated with reducing sperm count and motility (Khanan et al., 1986; Ahmed et al., 2002); therefore, it should be avoided if trying to conceive.

Some herbal remedies require fresh tulsi leaves to be chewed in order to obtain the medicinal effects. The plant contains natural mercury, which may be harmful to the teeth if the leaves are chewed and in prolonged contact with the mouth (Joshi et al., 2012). This can be avoided by swallowing the leaves whole with water or drinking infusions. If the plant is grown in areas where there are high levels of chromium contaminating the soil (i.e. from industrial leaks, fertilisers, and pesticides) it can have severe consequences as chromium can damage the liver and kidneys (Rai et al., 2004).

Singh et al. (2001) revealed the fixed oil of tulsi at a dose of 3ml/kg prolonged blood clotting time; they claimed the effect was because the oil prevented platelet aggregation. The blood thinning properties of tulsi have been compared to that of aspirin; therefore, people taking anti-platelets (e.g. aspirin and clopidogrel) and anticoagulants (e.g. warfarin) should consume tulsi with great caution (Singh et al., 2001). Winston and Maimes (2007) highlight that tulsi enhances cytochrome CYP450 enzyme activity. This can increase elimination of drugs rendering them less useful or ineffective. However, the American Herbal Products Association released an updated botanical safety handbook in 2013 which stated there were no drug interactions or adverse effects identified for tulsi (Gardner and McGuffin, 2013).

4.4.5 Botanical Identity of Tulsi

Typical characteristics of the Lamiaceae family include a square stem, opposite and decussate leaves, with numerous oil glands which secrete aromatic oils giving the plants distinct odours (Paton et al., 2005; Schmiderer et al., 2008). Part of the Lamiaceae family is the *Ocimum* genus, which has around thirty species (Paton, 1992). Alongside the traditional culinary basil (*O. bascilium* L.), tulsi (*O. tenuiflorum* L.) is the Indian alternative, commonly referred to as Holy basil; as reflected in its former Latin binomial *O. sanctum* L. which translates to sacred or the incomparable one. The word basil is likely to have originated from the Greek word *Basileus* which means kingly or royal. Some may refer to tulsi as the 'King of Herbs' however due to the Hindu association of the plant with the goddess Virinda Tulsi others call it the 'Queen of herbs' (Miller and Miller, 2003).

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Order: Lamiales
Family: Lamiaceae
Genus: *Ocimum*
Species: *O. tenuiflorum*
Botanical name: *O. tenuiflorum*
Synonyms: *O. sanctum*
(Hiran, 2009)

Tulsi can be described as a highly branched herb or shrub which can grow up to a meter tall, from the tiny round, dark brown, odourless but pungent tasting seed (Joshi et al., 2012). The bright green to dark purple, aromatic leaves are classically arranged in an opposite formation, with an oval-oblong shape similar to that of mint leaves. The square stem is hairy, purple to brown coloured, and woody in appearance; while the flowers can range in colour from white, yellow, and purple (WHO, 2002b).

There are a variety of different types of tulsi available (Figure 4-2). According to Ayurvedic scriptures the most common types being Raam tulsi, and Shyam also referred to as Krishna tulsi. Botanists classify these two types as *O. tenuiflorum;* and the less common type is Vana

tulsi (*O. gratissimum*) which is commonly known as African basil (Joshi et al., 2012). Although the plants are characteristically different, they may all be referred to and kept as tulsi (Maimes, 2004).

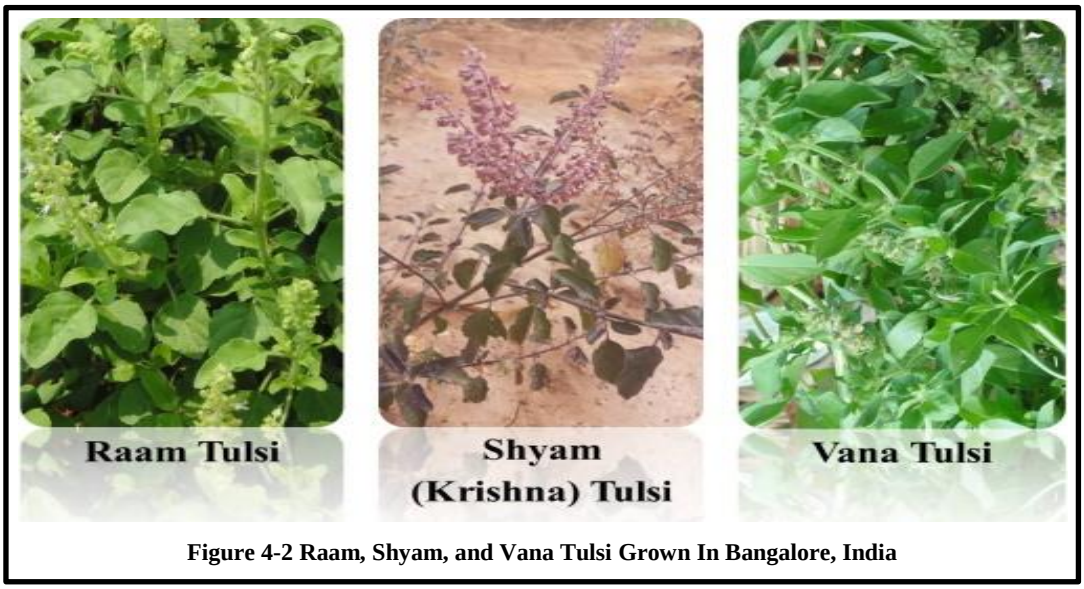


Figure 4-2 Raam, Shyam, and Vana Tulsi Grown In Bangalore, India

4.4.5.1 Microscopic Characteristics of Tulsi

WHO (2002) describes tulsi leaves as having a layer of small, quadrangular cells on the lower epidermis, trichomes of 2-6 cells, including short glandular trichomes, and diacytic stomata located on both the upper and lower epidermis. Similar studies by Datta et al. (2012) and Joshi et al. (2012) also recorded several important characteristics of tulsi including: oil globules, multicellular and unicellular simple and glandular trichomes (Figure 4-3).

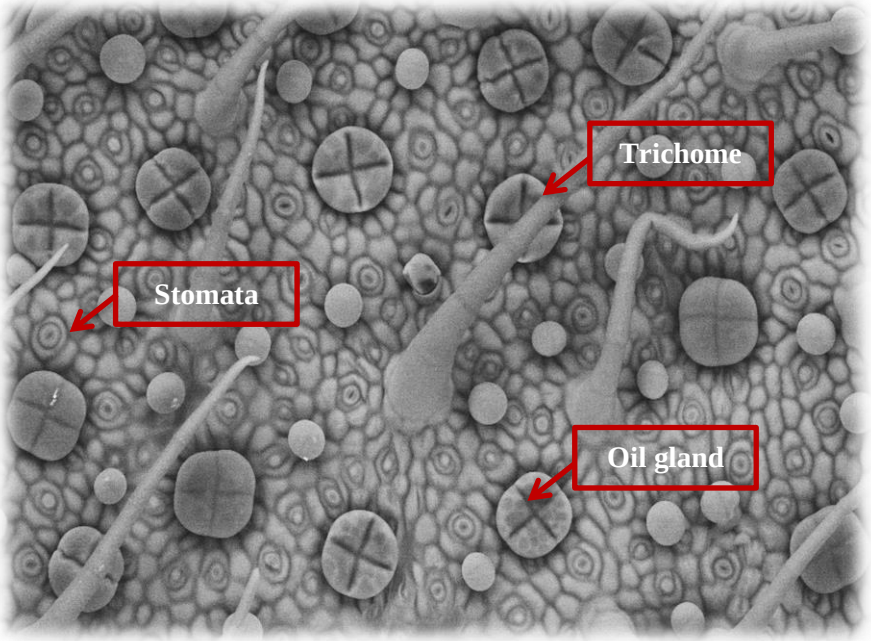


Figure 4-3 Image of the Lower Epidermis of a Tulsi Leaf at 500x Magnification under a Scanning Electron Microscope
Taken at De Montfort University by R. Armitage and S. Bhamra.

The image (Figure 4-3) reveals a high concentration of trichomes on the lower surface of the tulsi leaf. Trichomes often have several functions including: producing and storing essential oils and chemicals which may be used to supplement the defence mechanism of the plant, and offering protection to the leaf from insects and larvae (Levin, 1973). The structure, function, and density of trichomes can be used for taxonomic purposes. Numerous oil glands can also be seen in Figure 4-3. The Lamiaceae family has several species which are highly aromatic; most of the species produce and store essential oils in specialised epidermal oil glands (Schmiderer et al., 2008). The oils produced are distinctive of the species and can be used to identify the plants. The oils are composed of a mixture of compounds, which have many health benefits. The essential oils produced by tulsi contain the active compound eugenol, which has antiseptic and anaesthetic properties (Prakash and Gupta, 2005). The scent of the oil deters insects and has been used as a mosquito repellent (Joshi et al., 2012).

4.4.6 Methods of Plant Identification

The European Pharmacopoeia is a standard reference for the quality control of medicines (European Directorate for the Quality of Medicines & HealthCare, 2015); it has defined morphological and chemical profiles for medicinal plant species. Unlike traditional methods of plant authentication (i.e. morphology and chemical analysis) (Chen et al., 2014); DNA barcoding is a relatively new technique for plant identification, the applications of which are still being explored.

4.4.6.1 Morphology

A dichotomous key is used to sequentially identify parts of the plant to categorise the plants family, genus, and species (Jacono, 2009). Morphology encompasses the use of highly trained individuals who can identify the plants superficial structures to classify them; it includes the macroscopic and microscopic features. The macroscopic features are the physical characteristics of the plant such as the colour, size, structure, shape, taste, and aroma of the plant (Joshi et al., 2004). The microscopic structures of the plant can be seen using powerful microscopes and techniques such as scanning electron microscopy (SEM). The morphological features can be affected by the age of the plant, stage of harvesting, processing, and storage of the plants (Joshi et al., 2004). A clear example of how age can affect the microscopic features can be seen in Figure 4-4; the younger tulsi leaf has more trichomes which may help protect the leaf during development, in comparison to the mature leaf which has less trichomes.

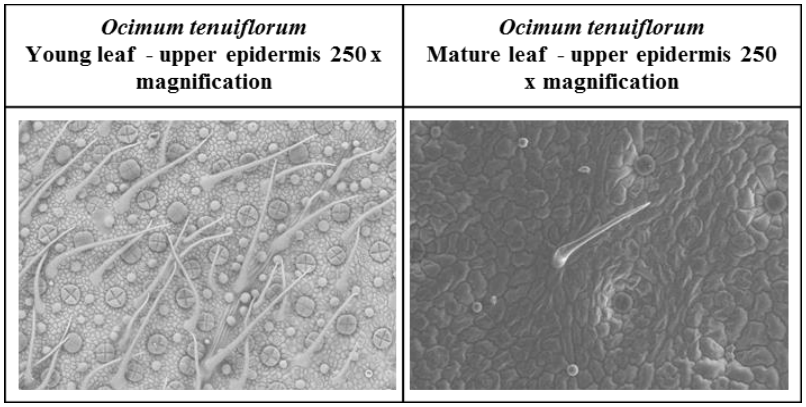


Figure 4-4 Image of Young and Mature *O. tenuiflorum* Leaf under a Scanning Electron Microscope
Taken at De Montfort University by R. Armitage and S. Bhamra.

SEM is a technique which can be used for examining plant surfaces at high resolution and to determine plant species. Electrons from a beam interact with atoms on the surface of the sample, producing an image of the surface composition. Previously, plant tissues needed to be preserved by dehydration for observation in an electron microscope otherwise the sample would be distorted under the high vacuum. Often samples had to be coated with conductive

material to prevent the accumulation of electrostatic charge, which could reduce the quality of the image produced. However, advances in technology mean the fresh sample can be analysed under SEM without altering the surface properties (Pathan et al., 2008). The technique also allows surface structures to be measured and quantified in three dimensions (Talbot and White, 2013).

4.4.6.2 Chemical Analysis

In the early days of botanical identification, the introduction of microscopy was a ground breaking and revolutionary technique used to aid the authentication of plants; later, advances in technologies promoted the use of spectrophotometry and chromatography (McCutcheon, 2002). Since then the chemical profile of plants has been used as a valuable tool for authentication, quality control, and standardisation of medicinal plants (Joshi et al., 2004). Thin layer chromatography (TLC), high performance thin layer chromatography (HPTLC), and high performance liquid chromatography (HPLC) are some of the techniques currently used for the identification of plants.

TLC is inexpensive, simple and fast to use; however, the poor reproducibility of results and lack of automation are major drawbacks of this technique (Dong, 2013). The refinement of HPLC and HPTLC techniques mean they are more precise, can be automated, and the results are reproducible (Cziczo, 2004; Dong, 2013). One of the main limitations associated with chemical analysis is that the chemical profiles can be affected by the natural variability of plants. This can be affected by the growing conditions (regional variations, soil, climate, and pests), stage of harvesting, drying, storage (treatment with pesticides and preservatives), and processing (McCutcheon, 2002; Joshi et al., 2004; Vlietinck et al., 2009).

Currently European Pharmacopoeial standards are based on a plants morphology (macroscopic and microscopic descriptions), and a combination of chemical analysis techniques, for example TLC and HPLC (McCutcheon, 2002; Vlietinck et al., 2009). The use of DNA authentication techniques to identify the plants species could also be used alongside the current standards (Joshi et al., 2004). The British Pharmacopoeia (2016) has recently validated the use of DNA based identification techniques for HMs.

4.4.6.3 DNA Authentication of Plants

DNA barcoding is a technique used to identify species based on short regions of DNA (Kress et al., 2005). Variations in a defined region of the DNA sequence can discriminate between different species, identify contaminants and adulterants (Joshi et al., 2004; De Mattia et al., 2011). This novel authentication technique was introduced by Paul Hebert in 2003 (Kress and Erickson, 2008). The analogy of a supermarket scanner identifying a product from the black and white stripes on the barcode was used to exemplify how this technique can be used to identify species by their unique genetic sequence (Kress, 2009).

To be able to distinguish between species, the target DNA barcode must contain enough variability, but, must also have regions of similarity for the universal primers (Rai et al., 2012). A primer is a short fragment of DNA which is complimentary to the target region of DNA. Plant DNA is stored in the mitochondria, nucleus, and chloroplasts. The mitochondrial cytochrome c oxidase gene (cox1) is highly conserved in plants; the low level of sequence variation renders this region ineffective for authentication (Kress et al., 2005). The internal transcribed spacer (ITS) region of the nuclear ribosomal unit is one of the most commonly sequenced regions in plants (Kress et al., 2005). It evolves relatively quickly and can be useful in determining interspecies and sometimes intraspecies relationships (Cullings and Vogler, 1998). The 5.8S rRNA gene evolves slowly, like the other genes for the 18S and 28S rRNA; and is often used as an alignment tool due to its location (Figure 4-5). Although, in certain groups the ITS region has reduced species level variability so may not be as useful (Kress et al., 2005). Several other concerns with the ITS region include: the existence of divergent paralogous copies within individuals, development of secondary structures which result in poor quality sequencing data, and the inadvertent amplification of endophytic DNA in the presence of fungal contamination (Kress et al., 2005). These limitations of the ITS region have led to it being rejected as a core component of the plant barcode (Hollingsworth et al., 2011).

Figure 4-5 The ITS Regions in the Nuclear Ribosomal RNA Repeat
Adapted from Cullings and Vogler (1998). ITS – internal transcribed spacer; IGS – Intergenic spacer. ITS-1 is located between the 18S and 5.8S ribosomal RNA gene; ITS-2 is located between the 5.8S and 28S ribosomal RNA gene.

The Consortium for the Barcode of Life (CBOL) have proposed two coding regions from the plastid (chloroplast) genome, *matK* and *rbcL*, as *"core barcodes"* for plants, to be used alongside other regions (Hollingsworth, 2011). The non-coding chloroplast *trnH-psbA* marker has also been identified as an efficient region for discriminating between species (Vassou et al., 2015). Kress et al. (2005) propose the *trnH-psbA* region is the best plastid option for sequencing as it has good priming sites, length, and interspecific variation. In addition, as the *trnH-psbA* region is shorter, it may be useful for damaged or degraded samples (Howard, 2010).

DNA analysis can be used as a stand-alone method or in conjunction with traditional methods for the identification of plant species (Chen et al., 2014). DNA barcoding is not restricted by the condition of the plant, as with morphological features which can change and degrade depending on various harvesting, processing, and storage conditions (McCutcheon, 2002). It does not require specialist taxonomic knowledge for identification. Unlike chemical analysis which can vary between plants harvested at different stages (young or mature) DNA analysis is not affected by the age of the plant (Kazi et al., 2013). The genetic composition is not affected by environmental factors or physiological conditions (Joshi et al., 2004). The amount of plant sample required is very small (from as little as 0.02g of dried or 0.1g of fresh plant material), in comparison to other techniques which may require several grams or a full plant for identification. DNA can be extracted from fresh, frozen, dried, or preserved samples. As the sample of DNA extracted is very small (usually 100µl) it is easy to store, compared to bulky plant specimens (Howard, 2010).

Nevertheless, there are several limitations with using DNA barcoding including: the amplification of non-target DNA from contaminants, and problems with primer specificity and amplification (Hollingsworth, 2011). Recently the New York State Attorney General Eric Schneiderman's office sent out cease and desist letters to four retailers (GNC, Target, Walmart, and Walgreens) to stop the sales of some herbal supplements sold in New York. As research commissioned by his office found products were substandard according to the DNA testing. They revealed that many of the products (79%) could not be genetically verified or found ingredients not listed on the label (Schneiderman, 2015); for example, Echinacea, Ginseng, and St John's Wort products from the various retailers (e.g. Walmart and GNC) were found to contain contaminants such as asparagus, rice, grass, wheat, and citrus. Schneiderman's results were questioned as he relied on just one technique for species authentication and all the work was conducted in one laboratory. Critics have said that his results should have been validated with microscopic or chemical methods, rather than being used as a stand-alone method for authentication. A statement released by the Chief Science Officer of the American Botanical Council (ABC) Dr Stefan Gafner, and ABC Founder and

Executive Director Mark Blumenthal stated, *"DNA testing seldom is able to properly identify chemically complex herbal* extracts *as little or no DNA is extracted in many commercial extraction processes"* (American Botanical Council, 2015). A major drawback to the use of molecular techniques is that the plants DNA may be damaged or degraded during manufacturing and processing stages; however, to try and overcome this problem, primers have been designed to target shorter fragments (Chase et al., 2005). Furthermore, the lack of reliable reference barcodes for species identification may be limiting the use of this technique. This is one of the key issues identified by this research and the aim is to help contribute some new barcodes to the references libraries.

The process of DNA authentication involves the extraction of DNA, a polymerase chain reaction (PCR), gel electrophoresis, and sequencing. After this a DNA sequence is generated which can be used to identify and authenticate plant species. The theory behind each stage will help to explain how DNA analysis is conducted and what happens during the processes.

- **DNA Extraction**

The extraction of DNA from plants (fresh or dried), tablets, capsules, tea bags, and other formulations can be done by using various protocols or commercial kits. Initially the sample needs to be lysed to disrupt the cell wall, cell membrane, and nuclear membrane to access the DNA. This can be done using chemical and physical methods (e.g. manual grinding with a pestle and mortar, or sonication). The DNA is then precipitated ensuring the removal of molecules such as proteins (i.e. using a protease), polysaccharides and lipids (i.e. using a detergent or surfactant), phenols, Ribonucleic acid (RNA) using an RNase and other secondary metabolites which may affect the quality of the purified DNA.

- **Polymerase Chain Reaction (PCR)**

A PCR needs to be carried out in order to exponentially amplify the target region of the DNA. The basic components required for a PCR include the DNA template, primers, DNA polymerase, deoxynucleoside triphosphates (dNTPs), and a buffer to maintain optimal conditions for the reaction. Once the reaction mixture is created, it is subjected to repeated cycles of variable temperatures for denaturing, annealing, and extension of the template DNA.

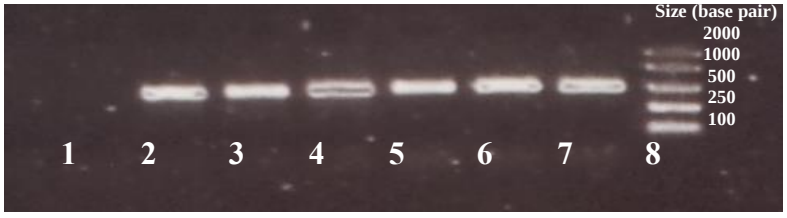
Primers are small fragments of DNA which act as a starting point for DNA synthesis. The PCR amplification requires two primers, which act as templates for sequence amplification

in the forward and reverse direction. The free nucleotides (dNTPs): Adenine (A), Cytosine (C), Guanine (G), and Thymine (T), are attached to the complimentary sequence of the primers template by the DNA polymerase enzyme, which assembles the new DNA strand. MyTaq Red Mix (2x) is a ready to use mixture for PCR reactions; it contains the buffer, DNA polymerase enzyme (Taq polymerase), dTNPs, $MgCl_2$, enhancers, and stabilisers to ensure a robust PCR reaction occurs (Bioline, 2015).

Once the PCR mixture is created, it is put in to a thermal cycler for the PCR reaction to commence. The temperature is initially raised up to 95ºC to enable the DNA double helix to be unwound, by breaking the hydrogen bonds; this exposes the single strands of DNA. The reaction temperature is then reduced for the annealing step; where the primers bind to specific sites on the single strand of DNA. The temperature is then increased for the final step of elongation, where the Taq polymerase synthesises a new complimentary strand of DNA by adding the free dNTPs. These cycles are repeated to amplify the template DNA. Finally, there is an extension period which ensures the reaction is complete.

- **Gel Electrophoresis**

Gel electrophoresis is the process of separating and analysing DNA fragments based on their size. An electric current is used to attract the negatively charged DNA fragments towards the anode. Shorter molecules move faster; therefore, they migrate further along the gel. A dye (e.g. SYBR® Safe) added to the gel enables results to be viewed under ultra-violet (UV) light in a transilluminator. A single bright band indicates the PCR has amplified the DNA (Figure 4-6). A faint band could indicate a low concentration of DNA in the sample. A smeared band could suggest too much DNA in the sample, DNA degradation or contamination (Palumbi et al., 2002). Multiple bands formed suggest multiple priming sites for the primer on the target DNA (National Diagnostics, 2011); in such cases the bands can be cut out and purified for analysis. If no band is seen it could mean the PCR failed, the quantity or concentration of the DNA template was insufficient, or the presence of contaminants, in such cases the PCR can be repeated after the original DNA sample has been cleaned. A DNA ladder can be used to determine the size of a DNA fragment. A positive control is a known sample which amplifies and is used to ensure the PCR reaction works; while, a negative control (distilled water) is used to ensure there is no contamination during the process.



Key:
1. Negative Control
2. Positive Control
3. Sample A
4. Sample B
5. Sample C
6. Sample D
7. Sample E
8. DNA Ladder

Figure 4-6 Image of a Gel, after PCR Amplification and Gel Electrophoresis of Several *Ocimum* Samples (A-E), Negative and Positive Controls, and a DNA Ladder
Negative control – distilled water; positive control – DNA sample known to amplify; DNA ladder – Bioline Easy Ladder 1.

- **Sequencing**

During the sequencing process a reaction similar to that of the PCR stage is carried out. Primers, DNA polymerase, dNTPs and dideoxynucleotides triphosphates (ddNTPS) are added to the query sample. The ddNTPS lack the 3 prime hydroxyl group required for extension, therefore these act as the terminators of the elongation of the DNA sequence. The ddNTPs representing each of the four nucleic acid bases (A, C, G, and T) are labelled with a different florescent tag which will help with detection. These ddNTPs attach complementarily to the new DNA strands; thus, DNA chains of varying length are produced (Figure 4-7).

Capillary electrophoresis is then used to sort the DNA fragments by length. The sample is loaded into a capillary tube which contains a viscous polyacrylamide gel. An electrical current is applied, which pulls the negatively charged DNA through the capillary; the shorter fragments migrate through the gel faster than the larger strands. As the fragments emerge at the end of the capillary they pass a laser beam, this causes the dye to fluoresce. The fluorescence emitted by each base is detected by a photocell which then relays the information into a computer that visualises the data as an electropherogram (Wiley, 2012). This creates the genetic sequence of the sample.

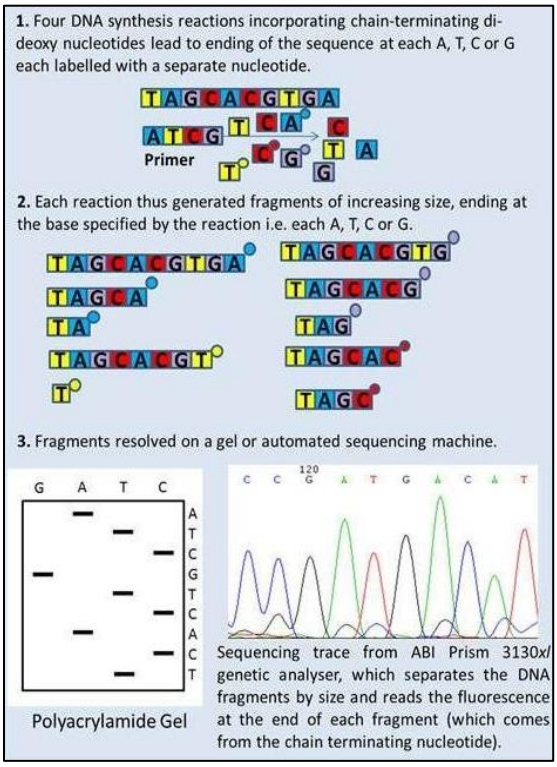


Figure 4-7 DNA Sequencing Summary, from Etheridge 2012

- **Species identification and authentication**

The Consortium for the Barcode of Life (CBOL) is an international initiative which aimed to develop DNA barcoding as a global standard for the identification of biological species. Genetic reference libraries have been created for sharing DNA sequences, including GenBank and the Barcode of Life Database (BOLD). These databases allow DNA sequences to be shared, managed, analysed, and used for comparisons (De Mattia et al., 2011; CBOL, 2015). Specimens used to create DNA barcodes still need to be authenticated by a taxonomist using the traditional methods of morphology to ensure the correct species is being used for the standard. Once a database is set up, fast and reliable identification can commence.

4.4.6.4 DNA Authentication of *Ocimum* Species

Several researchers have explored the use of different regions (e.g. nuclear ITS, plastid *matK*, *rbcL* and *trnH-psbA*) for DNA barcoding of the *Ocimum* species. Some have created reference sequences which are available on databases such as GenBank, while others have used the reference sequences to authenticate fresh, dried and commercial tulsi products.

Jürges et al. (2009) identified the difficulties of extracting tulsi DNA from multicomponent formulations and tried to define markers to distinguish between different *Ocimum* species. The ITS 1 and ITS 2 markers were amplified for eight *Ocimum* samples, of which five were supposedly *O. tenuiflorum*. Their results identified three of these samples were truly *O. tenuiflorum* while the other two samples had been incorrectly identified and were other *Ocimum* species instead. Their findings were confirmed by microscopic and macroscopic characteristics of the samples. One of the challenges Jürges et al. (2009) found was the different terminology used in Ayurvedic tradition and scientific botany. Although tulsi is the term used for *O. tenuiflorum* Jürges et al. (2009) claim in different parts of India "tulsi" could be used for other *Ocimum* species or any kind of basil depending on the origins. Their use of DNA barcoding to identify tulsi samples in commercial samples had limited success; however they were able to produce several reference *Ocimum* sequences.

Christina and Annamalai (2014) claim *Ocimum* species are morphologically very similar to one another hence easily misidentified. Consumption of the wrong species could have adverse effects or an inappropriate therapeutic effect. To address these issues Christina and Annamalai (2014) used DNA barcodes to validate several "*Medicinally important Ocimum species*." They looked at the efficiency of three barcodes (*matK*, *rbcL* and *trnH-psbA*) to identify nine *Ocimum* species from India. They concluded the markers they used showed a high PCR amplification efficiency which enabled the discrimination of the *Ocimum* species. Of the three markers used, they found the *trnH-psbA* region was ideal for the authentication of *Ocimum* species although it was not able to distinguish between the two varieties of *O. tenuiflorum* - Raam and Krishna tulsi.

Anbazhagan et al. (2014) collected four morphologically distinct plant samples which were identified as being *Ocimum* species. Using the *matK* gene for the PCR amplification they identified three of the samples were *O. tenuiflorum* and one was *O. basilicum* when the sequences were compared to sequences in GenBank. Like Christina and Annamalai (2014), Anbazhagan et al. (2014) also revealed that the *matK* region was suitable for DNA barcoding of *Ocimum* species.

The British Pharmacopoeia (2016) recently validated the use of DNA barcoding techniques for the identification of herbal medicines. They used *O. tenuiflorum* as an example to demonstrate how DNA barcoding could be used authenticate the plant material which may be used in herbal medicines. The *trnH-psbA* region was used to create the reference *O. tenuiflorum* sequence which is now part of the Holy basil monograph in the British Pharmacopoeia (2016).

This research highlights the applications of DNA barcoding techniques for authenticating *Ocimum* species.

4.5 Methodology

A mixed methods approach was used for this research; the use of semi-structured interviews and molecular techniques presented a dynamic approach to this multidisciplinary research. This section outlines the rationale for the methods used for this investigation.

4.5.1 Interviews with South Asian Communities and Sample Collection

Interviews are a type of qualitative research method which enables richer, in-depth knowledge of the individual participants' perception regarding the objectives of the study to be explored (Gill et al., 2008). A semi-structured interview process was used to collect primary data for this research. A mixture of open and closed questions were used during the interviews; as open ended questions give participants the opportunity to share as much information as they wish, while, closed questions enable quantitative analysis of results.

During structured interviews the researcher reads out the questions and records the respondents answers; they do not allow for deviations from the set questions (Moore, 2000). Whereas, semi-structured interviews give the flexibility to ask questions around the topic. If participants do not understand the question this type of research method gives the researcher the flexibility to explain the question in a way the participant understands. In addition, it gives the researcher the opportunity to explore interesting concepts further when they are identified (Wisker, 2008). This method allows the researcher to build rapport with participants; which in this research made it easier to obtain tulsi samples for molecular analysis from participants. Conducting semi-structured interviews may be time consuming and expensive, and the results may be difficult to analyse and generalise if no trends are observed; but, with the correct training and skills this is a valuable method of obtaining unique primary data (Moore, 2000).

The structure of the interviews was shaped by results from the SATMED questionnaire which the researcher was interested in exploring in more detail. The interview questions (Appendix 8) were designed to explore the cultural and commercial values of tulsi; a plant which many participants in the SATMED questionnaire claimed to use, grow, or import to the UK. An interest in establishing the country of origin of tulsi plants was addressed by asking participants where their seeds or plants were originally from. Participants were asked a range of questions to determine where their knowledge of the plant came from, and what they used it for. Participants were given the opportunity to express their views about the use of tulsi for religious and medicinal purposes, the growing conditions required, and any other special precautions to take with the plant.

An opportunity sampling technique was used to recruit participants who were willing to take part and give a sample of their tulsi plants. A participant information leaflet (PIL) was given to potential participants so they knew what the research was about and what was required of them (Appendix 9). Although this technique does not allow a representative sample to be selected, it is a fast and efficient way of recruiting a sample (McLeod, 2014). Participants were identified through the researchers' personal contacts; initially, recruiting friends and family who kept a tulsi plant, and were willing to participate. Followed by contacting participants who agreed to take part; either from previous contact with the researcher (during the SATMED questionnaire distribution), via the social media network created (Facebook), or by word of mouth from other participants. Posters and leaflets (Appendix 10 & 11) designed were used to create awareness of the project and provided contact details of the researcher for people interested in taking part. A website (www.dmutulsiproject.webs.com) and a Facebook profile (Tulsi Project - https://www.facebook.com/pages/Tulsi-Project/531956563523250?fref=ts) were also created to raise awareness of the project. The participant inclusion criteria for this aspect of the research was very broad; anyone over the age of eighteen years old, of SA origin, who had a tulsi plant or any tulsi products, willing to take part were accepted. (See Appendix 2D for the ethics proposal for this research).

Tulsi leaves, seeds, and other samples were collected from participants during the face-to-face interviews. Several interviews were conducted remotely via telephone; these participants were asked to post samples to the researcher. All tulsi samples collected from participants were classified as 'community samples' and given a code to identify where they were collected from (see section 4.5.2).

4.5.2 Collection of Tulsi Samples

A variety of tulsi samples were collected for molecular analysis. This included samples from participants taking part in the interview and any volunteers encountered throughout the research period; these samples were labelled as 'community samples'. Tulsi products (e.g. seeds, plants, tablets, capsules, oils, juices, soaps, and shampoos) bought from multiple sources in the UK, India and online were identified as 'commercial samples'. The 'reference specimens' represent the reference *Ocimum* samples obtained from botanists and established sources; i.e. Professor Peter Nick (Botanical Institute Karlsruhe Institute of Technology, Germany), Dr. Eike Reich (Director at CAMAG Laboratory, Switzerland), and The Royal Botanic Gardens Kew - DNA bank (samples from Chase M.W and Suddee et al.). A unique code for each sample was issued; a letter for the source/ location (e.g. A = Africa, B = Birmingham, C = commercial, G = Germany, I = India, L = London, Li = Leicester, V =

reference, etc.), followed by the chronological sample number (Appendix 12). For community samples given by participants who took part in the interviews the participant identification code was the same as the sample code for their tulsi sample. If multiple samples were given by a participant a letter was added to the end of the identification code (e.g. participant B15 from Birmingham gave two samples - B15a and B15b). The letter 'g' after a code was used to represent seed samples which were given from the original plant sample (e.g. B07 leaves from a plant and B07g were seeds from the same plant).

4.5.3 Scanning Electron Microscopy of Tulsi Samples

The Carl Zeiss Evo® HD 15 scanning electron microscope was used to conduct the SEM work on the tulsi samples collected, under the supervision of Rachel Armitage (Senior Technician and PhD Researcher) and Liz O'Brien (Senior Technician). Several of the reference *Ocimum* samples obtained from Professor Peter Nick were analysed under SEM.

4.5.4 DNA Analysis of Tulsi Samples

In order to authenticate a plant species the DNA must first be extracted, followed by a PCR to amplify the target DNA sequence, and then gel electrophoresis to check the PCR has successfully worked. If a positive PCR result is obtained the sample is then sequenced. The DNA sequence data can then be used to identify the plant species.

4.5.4.1 DNA Extraction

The Qiagen DNeasy Plant Mini Kit was used to conduct all DNA extractions. The Mini Protocol from the DNeasy® Plant Handbook, pages 22-25, was used (Qiagen, 2012).

- Once samples were selected for DNA extraction the appropriate amount was weighed (0.1g of fresh plant material or 0.02g of dried material). The samples were mechanically disrupted using a pestle and mortar and then lysed in a TissueLyser (for 1 minute at 30 Hz) with a tungsten bead in a microcentrifuge tube. For fresh plant samples 400µl of the lysis buffer (AP1) and 4µl RNase A (100mg/ml) was added during the maceration in the TissueLyser; while for dried samples these were added after. The tubes were then re-positioned in the TissueLyser and this step was repeated, to ensure equal homogenisation.
- For the cells to be completely lysed the samples were left to incubate at 65ºC for ten minutes.

- To precipitate the proteins and polysaccharides from the sample 130µl Buffer AP2 was added and then incubated on ice for five minutes.
- Cell debris and precipitates were removed by spinning the samples through a QIAshredder column, for two minutes at 14 000 revolutions per minute (rpm).
- 1.5 times the flow through volume of Buffer AP3/E was then added to help the DNA bind to the membrane of the DNeasy spin column.
- The sample was then applied to a DNeasy spin column and centrifuged for one minute at 8000 rpm.
- 500µl of Buffer AW was added and centrifuged (for one minute at 8000 rpm) and the flow through was discarded to remove any contaminants. This was then repeated; however, it was centrifuged for two minutes at 14000 rpm to dry the DNeasy membrane.
- For the final elution of the DNA, 100µl of Buffer AE was left to incubate on the DNeasy spin column membrane for five minutes, before being centrifuged for one minute at 8000 rpm. This was repeated, to leave a final volume of 200µl of pure DNA sample.
- Once the DNA was extracted 50µl was pipetted into two separate tubes to be stored at minus 20ºC and minus 80ºC for preservation. The 100µl left in the original tube was used as the working sample of DNA.

4.5.4.2 Polymerase Chain Reaction (PCR)

A master mix containing the MyTaq Red Mix (2x), forward primer, reverse primer, and distilled water was made (Table 4-1). 48µl of the master mix was aliquoted into a 0.5ml PCR tube, followed by 2 µl of template DNA. A positive control (known DNA sample) and negative control (distilled water) were always used to ensure the PCR had worked successfully and there was no contamination.

Table 4-1 PCR mixture with BioLine MyTaq Mixture

Component:	Volume per reaction:
MyTaq Red Mix	25 µl
Forward Primer	1 µl
Reverse Primer	1 µl
Template DNA	2 µl
Distilled Water	21 µl
Total	**50 µl**

The PCR tubes were then transferred into the Bio-Rad PTC-200 Thermal Cycler for the PCR reaction to commence.

Primers and protocols used include:

rbcL

*rbcL*_f (Forward primer) 5'-ATGTCACCACAAACAGAGACTAAAGC-3'
*rbcL*_rev (Reverse primer) 5'-GTAAAATCAAGTCCACCRCG-3'

PCR protocol for *rbcL*:
Initial denaturation step - 5 minutes at 95°C. Followed by 35 cycles of 30s at 95°C, 20s at 52°C and 50s at 72°C. Final extension period of 5 minutes at 72°C.

matK

3F Kim (Forward primer) 5'-CGTACAGTACTTTTGTGTTTACGAG-3'
1R Kim (Reverse primer) 5'-ACCCAGTCCATCTGGAAATCTTGGTTC-3'

PCR protocol for *matK*:
Initial denaturation step - 5 minutes at 94°C. Followed by 5 cycles of 30s at 94°C, 40s at 44°C and 40s at 72°C. Then 30 cycles of 30s at 94°C, 40s at 46°C and 40s at 72°C. Final extension period of 3 minutes at 72°C.

ITS

ITS1 (Forward primer) 5'-TCCGTAGGTGAACCTGCGG-3'
ITS4 (Reverse primer) 5'-TCCTCCGCTTATTGATATGC-3'

PCR protocol for ITS:
Initial denaturation step - 7 minutes at 95°C. Followed by 30 cycles of 1 minute at 95°C, 30s at 60°C and 1 minute at 72°C. Final extension period - 7 minutes at 72°C.

trnH-psbA

psbA (Forward primer) 5'-GTTATGCATGAACGTAATGCTC-3'
trnH (Reverse primer) 5'-CGCGCATGGTGGATTCACAATCC-3'

PCR protocol for *trnH-psbA*:
Initial denaturation step - 5min at 95°C. Followed by 35 cycles of 1 minute at 95°C, 30s at touchdown temperature* and 1 minute at 72°C. Final extension period of 7 minutes at 72°C.

*Touchdown temperature begins at 58°C, reduced by 1°C per cycle until 48°C, then continued at 48°C for the remainder of the program.

(Primers and Protocols obtained from Biomolecular Technology Group, A guide to molecular techniques, Standard Operating Procedures, 2013)

4.5.4.3 Gel Electrophoresis

After the PCR reaction was complete, the samples were loaded (5 µl) on a 1% w/v agarose gel (0.5g agarose powder mixed with 50ml 0.5 X TBE* buffer, and 2µl SYBRsafe™ as the DNA stain), with TBE buffer for the electrophoresis. The gel electrophoreses was run for 30 minutes at 90V. An image of the gel was then taken using the Bio-Rad Gel Doc EZ imager and the Image Lab™ Software. The Bioline Easy Ladder 1 was used to determine the molecular weight of the DNA.

**TBE = Tri-borate-EDTA, made by mixing 10.8g Trise base, 5.5g Boric acid, 0.584g EDTA and 1 litre of distilled water.*

4.5.4.4 Extraction of Multiple Bands

When multiple bands were seen on the agarose gel, the Qiagen® QIAquick gel extraction kit was used to cut multiple DNA bands from the gel for further analysis. In order to extract the DNA from the multiple bands, the gel electrophoresis was repeated with the following modifications: up to 50µl of PCR sample was loaded on the gel and it was run for 60-90 minutes at 90V. An image of the gel was printed to map the bands on the gel. The gel was then taken to the UV light box where the individual bands were carefully cut out using a blade. The segments were placed in a labelled tube before the protocol for the extraction was followed from the QIAquick Spin Handbook, page 25-26 (2006).

Once the DNA was eluted from the gel, another agarose gel was made to check the DNA had been successfully extracted, before sending the samples for sequencing. 5µl of the sample was mixed with 2µl of loading buffer before being pipetted onto the gel, which was run for 30 minutes at 90V. Samples were then sent for sequencing, or a PCR was done to amplify the DNA before sending the samples for sequencing.

4.5.4.5 DNA Isopropanol Clean Up

DNA may need to be cleaned in order to remove impurities and contaminants such as enzymes, proteins, and cell debris if they are not fully removed during the final stages of the DNA extraction. The isopropanol clean up method from the De Montfort University - Biomolecular Technology Group, A Guide to Molecular Techniques, Standard Operating Procedures, page 6, 2013 was used:

50µl of the DNA sample was added to 35µl of isopropanol in a 1.5ml microcentrifuge tube. The mixture was centrifuged at 15,000 × g for 30 minutes at 4°C. The supernatant was then

removed before re-suspending the pellet in 200µl of 70% ethanol. This mixture was then centrifuged at 15,000 × g for 10 minutes at room temperature. The supernatant was once again removed, and the ethanol was allowed to evaporate (for 20-30 minutes). Finally the pellet was re-dissolved in 50µl of elution buffer TE.

4.5.4.6 NanoDrop Spectrophotometry

The NanoDrop™ Lite (Thermo Fisher Scientific Inc) was used for quantification of DNA in the samples. It is a UV spectrophotometer which uses Light Emitting Diode (LED) technology to measure the samples absorbance at 260 and 280 nm. A very small quantity of sample (1-2 µl) is required for the analysis. It provides three key readings: concentration (ng/µl), A260, and A260/A280 purity ratio. The concentration value reveals the concentration of nucleic acid in the sample. The A260 measures the absorbance and should have a value between 0.1-1.0, results less than 0.1 are not very reliable. The A260/A280 purity ratio determines the level of protein contamination; a value of 1.8 is accepted as being pure DNA, lower values indicate the presence of proteins, phenols, and other contaminants which also absorb at 280nm (Thermo Fisher Scientific Inc, 2012).

4.5.4.7 Sequencing

Samples which gave a positive PCR result (as indicated by the gel electrophoresis) were sent for sequencing to an external sequencing company, Macrogen Europe, Amsterdam (The Netherlands) who provide Sanger sequencing services. The results from the sequencing company provided details of the sequence length, GC%, and also the sequence trace (electropherogram). The DNA data obtained were then used for analysis, identification, and construction of a reference database.

4.5.4.8 Sequence Analysis

Data was analysed using CLC Main Workbench 6 (CLC bio). This software enabled sequence data to be analysed by assembling DNA sequences, trimming sequences, producing contigs (i.e. overlapping DNA sequences to produce a consensus sequence), and multiple alignments. Default settings were used to produce contigs with an automated vote based conflict resolution. If sequences could not be assembled using the default settings, the minimum alignment read length was reduced from the default 50 bases to 1 base and the alignment stringency was modified from medium to low (as recommended in the CLC user manual).

The Basic Local Alignment Search Tool (BLAST) was used to input the query DNA sequences, for comparisons with reference DNA barcodes from reference databases for identification (http://blast.ncbi.nlm.nih.gov). DNA databases such as Genbank (http://www.ncbi.nlm.nih.gov/genbank/) and BOLD (http://www.boldsystems.org) were used to search for reference *Ocimum* sequences, which were then compared with query sequences in CLC.

4.6 Results and Discussion

4.6.1 Tulsi Project Interview Results

Semi-structured interviews were designed to collect information about the current use and importance of tulsi to individuals who kept the plant. Questions to understand why tulsi was kept, where it was originally from, and why it was important to participants helped to reveal some interesting information. While the interviews were conducted participants were asked for a sample of their tulsi plants, seeds, or any other tulsi products they had. These were then subjected to DNA analysis.

4.6.1.1 Sample Description

An opportunity sampling technique was used to recruit participants. Participants known to the researcher who had a tulsi plant were approached and asked if they would take part in this research. They were asked to answer some questions during a short interview (Appendix 8) and provide a sample of their tulsi plant, seeds, or any other tulsi products they had. Participation of one contact led to the recruitment of several others, leading to a snowball effect. Interviews were conducted with people from several locations across Birmingham, Leicester, and London. The awareness of the project raised via the social media Facebook page and the website led to people from Australia and Singapore getting in contact to see if they could take part and have their samples authenticated. Samples were posted and received from Singapore, however due to fungal contamination they were discarded.

Although twenty six tulsi samples were collected from participants, not everyone was able to take part in the interview. In total thirteen interviews were conducted with the collection of a tulsi sample for genetic testing. Most of the interviews were conducted face to face; while, some were completed via telephone and samples were then posted to the researcher.

All participants were of Indian ethnicity and over 30 years old. There were more female (77%, n=10) than male (23%, n=3) participants in this survey population (Figure 4-8). One participant was of the Sikh faith while all the others were Hindu. Just one participant was born in the UK, whereas most of the others were born either in India (n=8) or Africa (n=4). All of the participants' parents were born in India or Africa.

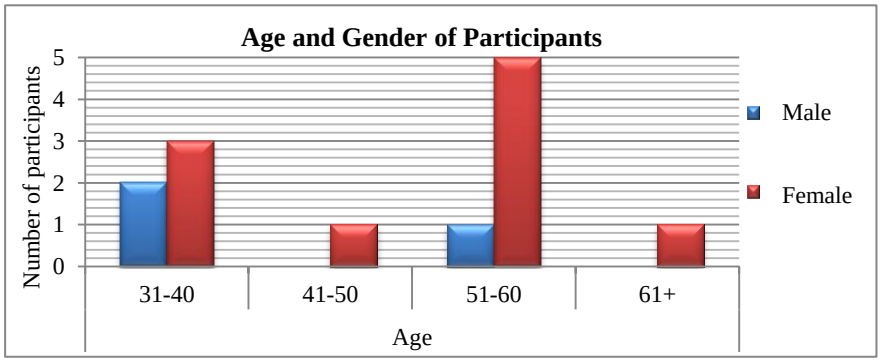


Figure 4-8 Participants' Gender and Age

4.6.1.2 Analysis of the Interviews

Participants were asked a series of questions to establish where they got their tulsi from, what they used it for, and to gather some background information for this research. As a semi-structured interview style was used, it gave the researcher the freedom to ask the questions in an informal conversational manner.

❖ ***Where did you get your Tulsi from?***

Many participants (54%, n=7) claimed to get their tulsi from their family; seeds and plant cuttings were often passed on from mothers, grandparents, or aunties. Some participants said they got their tulsi from friends (23%, n=3); while others (15%, n=2) said they got their tulsi from the temple (*mandir*), due to the religious affiliation tulsi plants can always be found in Hindu temples. One participant claimed she got her tulsi from both friends and family. It is common practice to share cuttings and seeds with friends and family. Some participants claimed tulsi seeds and dried material from India and Africa were brought to the UK during visits to family or via post (Figure 4-9). The migration pattern of people from India to the UK and Africa, and then from Africa to the UK can be seen in Figure 4-9; whether or not this impacts the species of tulsi found in the UK will be considered later.

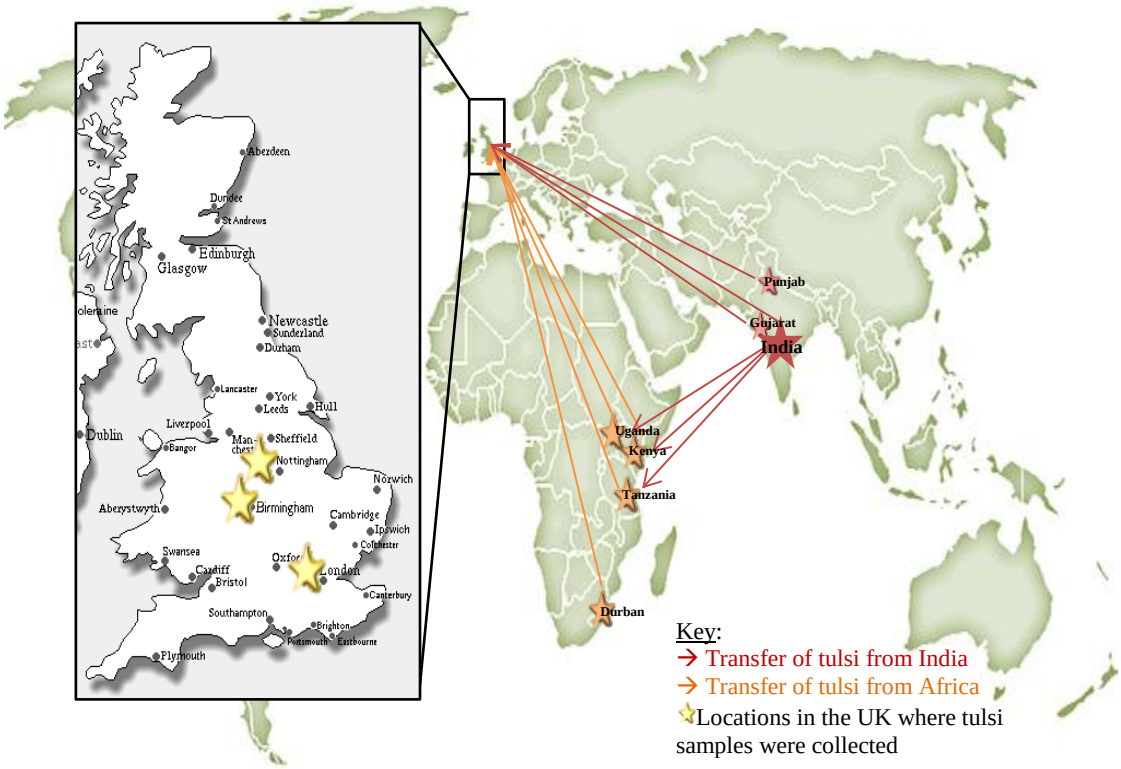


Figure 4-9 Map of Where Tulsi Samples Were Collected in the UK, and Where Samples Might Have Originally Came From

The map summarises where participants may have got their tulsi from and where samples were collected in the UK. This is based on where participants' families were in India and Africa and where participants or their families lived before migrating to the UK.

❖ ***Do you have any Tulsi seeds?***

Participants were asked if they had any tulsi seeds to uncover the source of their plants; in addition, the researcher was keen to collect seed samples to grow at the university. The vast majority of participants (77%, n=10) did have seeds; whereas, the others (n=3) said they got their tulsi as a plant or a cutting and did not have any seeds. Some participants claimed the original source for their seeds was either India or Africa. Seeds which had been taken from India to Africa and then to the UK were also identified by one participant (participant B06); she was born and brought up in Uganda but said her mother got her tulsi seeds from her grandmother in India, her mother sent her some seeds in the post when she moved to the UK where she kept her plant for sixteen years before it died. She now has another plant from a friend in Leicester and each year she harvests the seeds and shares them with neighbours, friends, family, and the temple.

❖ ***Do you grow your own Tulsi?***

This question aimed to establish whether or not people grew their own tulsi plants; as not all the samples obtained were fresh plant material. Twelve participants (92%) said they either did or had tried to grow a tulsi plant, just one participant said she did not grow her own tulsi; participant A14 said, "*No I do not grow it personally but my mum and mother-in-law do*."

Some participants said they had problems propagating the seeds and maintaining a healthy plant. While others claimed they tried to grow a tulsi plant but it did not grow very well here in the UK. For example participant B102 claimed, *"In India it is much easier to grow tulsi, but here in the UK it always dries out!"* These participants made reference to the size and abundance of the tulsi plants their families grew back in India and Africa. Participants commented on how the environment and climate in the UK was not suitable for growing tulsi. It was also noted that participants claimed Raam tulsi was easier to grow than Shyam tulsi. Those who were able to successfully grow their own tulsi plants said they kept it in a warm place near a radiator, a window to enable plenty of light to get to the plant, or in a porch to mimic a greenhouse. Many participants made reference to how difficult it was to keep tulsi; those who were able to grow and maintain the plant were believed to be very fortunate.

- ***Are there any special requirements for storing/ growing/ keeping Tulsi? If so what are they?***

The significant majority of participants (85%, n=11) claimed there were special growth requirements for ensuring the survival of their tulsi plants; such as participant B105 who said*, "The tulsi plant must be very well respected and cared for. You cannot touch it without washing your hands or ladies if it is the time of month."* Participants who were able to grow a plant, as well as those who were unable to keep tulsi mentioned several requirements for keeping the plant.

Most importantly participants identified the warm and sunny environment required for tulsi to flourish in, which were not ideal in the UK. As the tulsi plant is found predominately in the tropics it is accustomed to warm, humid and sunny climates (Grayer, 2001). In the UK, often the temperature is not optimal for growth of tulsi and during the winter months there are insufficient levels of sunshine. Due to the warm climates in Asia and Africa tulsi grows wild almost anywhere the seeds are sown. The minimum temperature tulsi will survive in is around 18ºC; hence, in the UK where temperatures often fall below this minimum requirement, tulsi must be kept indoors or in a greenhouse with the temperature maintained between 18-23 ºC. The use of artificial heating sources such as radiators were used to try and keep plants warm, but there was a risk of the plant drying out and dying if kept too close. Participants explained how difficult it was to keep their tulsi plants warm enough, in an area with plenty of light; a window sill in the kitchen appeared to be the most common place to keep tulsi.

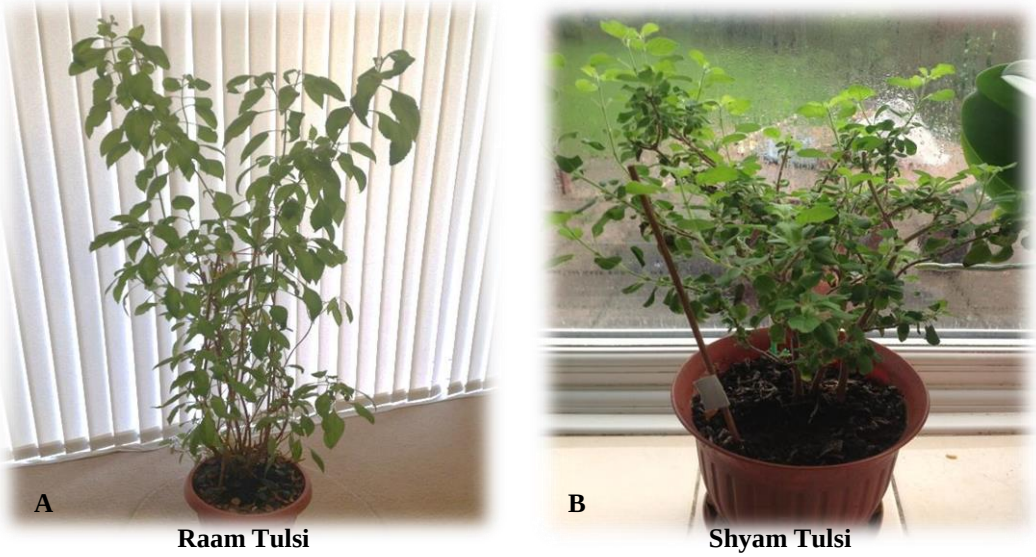
In India and Africa as the climate is well suited for tulsi to thrive it is often grown in the centre of the courtyard, as it is supposed to bring the family together and be a blessing. Some believe the plant should be kept in the front of the house to welcome the Gods, while others say it should be kept in the centre of the home. Participants had different views about where the tulsi plant should be kept; but, one thing which was consistent was that it should not be moved once placed.

The small dark brown seeds should be sown around April-May, covered lightly with finely sieved compost, and not over watered. In theory, the soil will need some extra nourishment as the tulsi begins to flourish, using plant feeds, fertilisers or manure can help the plant grow healthier and stronger (Wagner, 2010). However, participants made specific references to not using fertilisers, chemicals or even plant feeds as they would not be suitable for the holy plant. Participant B02 said, *"Tulsi should only be given water as it does not need anything else, it would be disrespectful to pour chemicals and fertilisers onto the goddess"*.

Traditionally the tulsi plant is idolised as a Goddess, Hindus believe extra affection should be shown to the plant. Special rituals and prayers (*poojas*) have been conducted for centuries to help the plant thrive. Wagner (2010) describes multiple ways of caring for and worshiping the plant, including: chanting the tulsi *Mantras*, saying the one hundred and eight sacred names of tulsi, gently kissing the leaves, and thanking the plant for being with you. During the interviews it become apparent that there were several 'terms and conditions' attached to the successful growth and longevity of the plant. It was noted that the plant should not be disturbed in the evening as it sleeps just like humans do, during menstruation women are not supposed to touch the plant as this is a time of impurity, the plant should only be watered or touched after washing your hands, and the plant should not be moved around as this would be classed as being disrespectful to the Goddess. The myth is that tulsi will only grow in fortunate people's homes, *''only the lucky people can grow this plant in their home*,' Participant B05.

❖ ***What type of Tulsi is this?***

Participants were asked to specify the type of tulsi they had. Some participants did not know the type of tulsi they had while others were able to differentiate between the different varieties of tulsi they had or knew of. The two main types of tulsi identified by the research population were Raam and Shyam tulsi (Figure 4-10). Raam tulsi was described as having large bright green leaves; the plant often grew very big and survived the harsh UK climate. On the other hand, Shyam tulsi was known to have smaller, darker green leaves and did not grow as tall as Raam tulsi. Some participants claimed their Shyam tulsi plants dried out during winter but rejuvenated during the summer time, while others said once their plant dried out in winter it never grew back. This suggests the Raam tulsi grown by participants may be a different species which does not require the same growth conditions as the Shyam tulsi. Six of the participants interviewed were not sure about the type of tulsi they had.



Raam Tulsi **Shyam Tulsi**

Figure 4-10 Community Raam and Shyam Tulsi Samples Collected During the Interviews

Photographs of tulsi plants in participants' homes identified by participants as A –Raam tulsi, B - Shyam tulsi.

❖ ***Can you tell me all the different names Tulsi has:***

With the wide spread use of tulsi across the world there are numerous ways of referring to it. This question was asked to be able to get a better understanding of how to refer to tulsi the correct way. Participants gave a range of names for tulsi including: *tulasi, Virinda, tulsi maa, tulsi devi, tulsi rani, Nandani, Laxmi,* and sweet basil. Many of the names given to tulsi have a religious connotation. The names given symbolise the religious significance for participants i.e. *'maa'* meaning mother, '*rani'* translating to queen, and *'devi'* referring to goddess. In Hinduism there are 108 names for tulsi; some devote worshipers believe by chanting the 108 names to the plant helps to promote its growth and prosperity. Other common names for tulsi include: holy basil, monk's basil, sacred basil, green tulsi, garden balsam, *solasi, sulasi, sursa, thulasi, tulashi, tjlsi,* and *Virandra* (WHO, 2002b; Joshi et al., 2012). In the various states of India the plant is referred to in different ways. The incorrect term or pronunciation could lead to great confusion; as experienced by the research team during field research in India. In areas where Punjabi or Hindi are spoken the word *'tulsi'* is used; however, in South India the term tulsi was not recognised but instead it was known as *'thulasi'*.

❖ ***Do you keep Tulsi in your home? If so why?***

All participants interviewed said they kept tulsi in their home; those who struggled to grow the plant said they kept it in their family homes in India and Africa. The main reason of keeping tulsi for 92% of participants (n=12) who were Hindus was the religious significance. Participants claimed the plants growth and presence in the home symbolised luck and it was a blessing to be able to keep it. Participant B15 said, *"It is very important if you are Hindu to keep tulsi,"* while participant B12 claimed, *"we keep it in our home for good luck and for religious ceremonies."*

The importance of having tulsi for offerings to God during *poojas* was mentioned. Participant B03 described how her mother used to make her take blessings from the tulsi plant kept in the centre of the courtyard, in their home in India, before any exams and important events. Just one Sikh participant (B01) who got her tulsi from a Hindu friend said she kept the plant for the health benefits associated with it. Participant B01 had diabetes and was told to take tulsi leaves in her tea to help reduce her blood glucose levels.

❖ ***Where did you learn about Tulsi?***

Participants were asked how they had learnt about tulsi to establish where and how this traditional knowledge was being transmitted. The religious and medicinal values associated with the tulsi plant were evidently passed on through generations, as the family appear to be the main source for learning about tulsi, for 62% (n=8) of participants (Figure 4-11). Due to the religious significance of the plant for Hindus, religion has also had a key role in transmitting the knowledge of the benefits of tulsi for three participants. Participants spoke about how the priest (*pandit*) talked about tulsi during religious ceremonies to explain the importance of the plant which represented the goddess Virinda Tulsi. Participants also shared their knowledge of what the religious significance was and what it meant to them. Some participants (n=2) claimed to have learnt about tulsi from their friends, while discussing natural remedies for various ailments. Participant B02 made reference to having learnt about tulsi during her education in India.

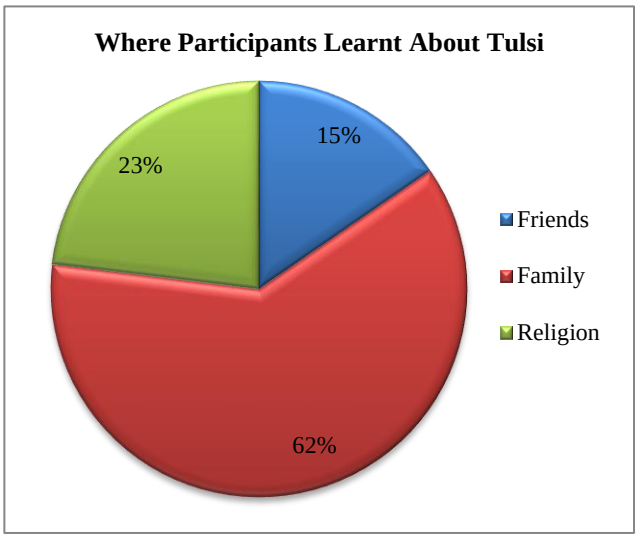


Figure 4-11 Where Participants Learnt About Tulsi

Participant B06 talked about her experience with tulsi growing wild in Africa; saying it grew in abundance on the sides of the streets and roads; she said her mum always talked about how important the plant was and felt like it was not being given the correct respect in the wild conditions it was seen in.

❖ ***Does Tulsi have a religious purpose in your life? If so what is it?***

As 92% of participants were Hindu, the tulsi plant did have a religious significance for them, even for those not actively practicing their religion. The one non-Hindu participant (B01) said, *'I am not Hindu but I still respect the plant as my friend has told me what the plant represents.'* Many participants detailed the reasons for worshiping the plant and explained why the plant was referred to as a goddess. Participants mentioned how it was used as offerings as *prasad* during prayers.

The purity of the plant was associated with the numerous healing properties, believed to be Gods cure for all diseases. The leaves placed in the mouth of the deceased were believed to

aid the passage to heaven. If the plant grows it is believed to symbolise a blessing and is perceived as being lucky. Due to the religious affiliation of the plant every *mandir* should have a tulsi plant; this makes it accessible for everyone to get blessings from the tulsi goddess even if they cannot keep a plant at home.

Many of the participants who kept tulsi for the religious properties were strict vegetarians and mentioned how the consumption of meat and alcohol should be avoided in the house where tulsi is kept, as it would be disrespectful to the goddess. One participant spoke about her friend who kept a plastic tulsi plant in her home so she always had the presence of the goddess with her, just like with other religious pictures and statues in her personal *mandir*.

- ***What do you use Tulsi for?***

By asking participants what they used tulsi for, the current use amongst SA communities in the UK could be established. Many participants (n=9) claimed to keep tulsi just for religious purposes. The plant was kept in the home to represent the goddess Virinda Tulsi and used during prayers as an offering to the Gods. During a research visit to a commercial tulsi supplier in Bangalore, India (April 2014), the problem of tulsi sales and waste were highlighted. Hinduism is the main religion in India (Census of India, 2011), with over 80% of the population identified as being Hindu. The demand for tulsi plants and leaves to be used as offerings to the gods during prayers and religious ceremonies is a big business. Therefore, tulsi needs to be quickly and widely available. Once the plants and leaves have been used for the religious ceremonies they are usually discarded as waste. Dr Velusamy Sundaresan and his researcher team at the Central Institute of Medicinal and Aromatic Plants, Bangalore, have tried to explore the possibilities of recycling the vast amounts of tulsi which are being wasted; however, progress has been slow and issues with authentication have been highlighted (Sundaresan, 2014).

- ***What are the medicinal benefits of using Tulsi?***

The significant proportion of participants (87%) were aware of the diverse range of health conditions for which tulsi could be used. Participant B02 made reference to tulsi being the cure to all conditions as it was a God given plant; this opinion was shared by many others who were aware of both the religious and medicinal values of the plant. Participants listed the use of tulsi for: asthma, chest infections, sore throat, cough, colds and flu, fever, diabetes, skin conditions (e.g. eczema and itchy skin), mouth freshener, excessive stomach acid production, and period pains.

For many of the conditions the most common way to consume tulsi was by infusing it in warm water or tea. For conditions such as cough, sore throat, and the use as a mouth freshener - one or two leaves could be chewed. For skin conditions participants recommended bathing in tulsi infused water or rubbing the leaf directly onto the affected area of the skin.

A small minority of participants (n=2) did not state any medicinal benefits of tulsi as they said they did not know any; they said they only knew the religious uses and had never heard about it being used as medicine.

- ***Is there any other information which you think is important/ interesting to know about Tulsi?***

Participants were given the opportunity to share any additional information about tulsi. Table 4-2 outlines the comments participants made, including some of the 'terms and conditions' to keeping a tulsi plant.

Table 4-2 Participants Comments about Tulsi

Participant	Comments
B02	*-The only thing tulsi maa needs is water. No artificial food, chemicals or fertilisers are needed.* *-If I have a cough I just take a few leaves and chew them.* *-Keep the tulsi in a warm place; I keep mine near the radiator.*
B03	*-Tulsi mala has 108 beads made from the woody stems of the tulsi plant. It is used to help you concentrate when you pray.* *-Tulsi should be kept in the centre of the home.*
B04	*-Do not touch tulsi on a Sunday.* *-If you keep the seeds you must do the plants marriage.* *-Can make holy water blessed with tulsi.*
B05	*-Tulsi represents lord Krishna so I tie a rakhi (cotton thread) on the plant, as I do not have any brothers of my own.* *-The plant does not survive in all homes only the lucky people can grow this plant in their home.* *-Cannot pick leaves in the evening.*
B06	*-Cannot keep it outside as will not grow so have to keep it inside the house.*
L10	*-If a lady is on her period and she touches or waters the tulsi, it will die.* *So do not touch the plant during this time.*
B102	*-It is a very important plant to my family – it is maintained very well and my parents nurture their plants. As it has a religious purpose it is very valuable for us.*
B105	*-It's a very sacred plant for us; it means you're very lucky if it grows.*

These comments illustrate the religious significance of tulsi and the traditions associated with keeping the plant which are evidently closely followed by communities in the UK at present.

4.6.1.3 Summary of The Interview Results

The interviews have helped gain a deeper understanding of why tulsi is so important to people and why it might be kept in the home. Evidently, a strong religious affiliation for Hindus means the plant has a special place in many of the participants' homes; although, this may not be the only reason for keeping tulsi. The numerous health benefits associated with tulsi may also be a reason for keeping the plant. Family, friends, and religion all have a role in transmitting the knowledge of the uses and benefits of tulsi. The 'terms and conditions' attached to the prosperous growth of the plant may just be a myth; but, they still have a strong precedence amongst SA communities in the UK today. The results have revealed that tulsi is culturally, medicinally and commercially an important plant. Several types of tulsi samples were collected from participants. As different varieties are available, the importance of selecting the correct species will now be explored by looking at the DNA of the plant species.

4.6.2 DNA Authentication of Tulsi Samples

The following section details the findings from the DNA analysis. It will describe how samples were collected and classified, some of the challenges faced during the study, and the achievements of using this novel molecular technique.

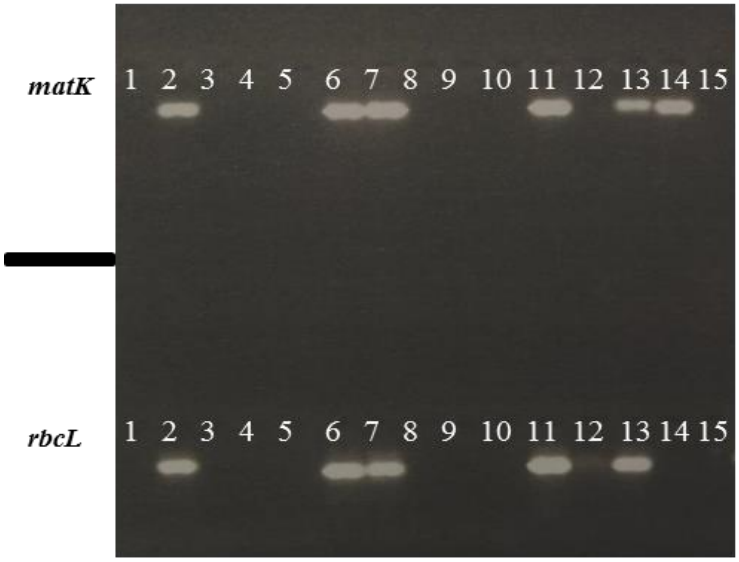
4.6.2.1 Classification of Tulsi samples

A total of 111 tulsi samples were collected through the duration of this research (Appendix 12). The samples collected were categorised as community, commercial, reference, or miscellaneous; 26 community samples were collected from participants encountered throughout the research period who donated their personal tulsi plants, leaves, or seeds. Forty-seven commercial samples included products such as teas, tablets, capsules, soaps, shampoos, creams, and oils, these were bought from shops, markets, and online; in the UK and abroad. Raw tulsi material ready to be used in commercial products which was obtained from Pukka Herbs, was classified in the commercial samples sub-group. Thirty-three *Ocimum* samples obtained from sources where the plants had been authenticated by expert botanists formed the 'reference' specimens. Six samples which were not identified as community, commercial, or reference were labelled as miscellaneous; these samples were collected by the researcher from various places while travelling throughout the research period.

Of the total 111 samples collected, 29 samples were excluded from the analysis as the quality of DNA of the sample was very poor and in many cases no DNA was isolated during the extraction process (Appendix 12). Two of the samples were seeds which failed to germinate on numerous occasions; a DNA extraction from the seeds was attempted, but was unsuccessful. It is difficult to extract DNA from seeds due to the high level of primary (polysaccharides and lipids) and secondary metabolites (e.g. in the case of Lamiaceae, caffeic acid and other polyphenols), as well as proteins, which can degrade the quality of the DNA (Hassan et al., 2012). These samples did not give a positive PCR result and the lack of DNA in the sample was confirmed by results from the NanoDrop analysis. A potential reason why DNA was not extracted from the samples could have been due to the types of products used, e.g. soap, syrup, capsules, and oils; in which the DNA may have been degraded during manufacturing processing.

4.6.2.2 The plastid *matK* and *rbcL* Regions

The Consortium for the Barcode of Life (CBOL) Plant Working Group, proposed two coding regions from the plastid genome – *matK* and *rbcL* as *''core barcodes''* for plants (Kress et al., 2005; Chase et al., 2005; Hollingsworth, 2011). Thus, initially the *matK* and *rbcL* regions were selected for PCR amplification and DNA analysis of the *Ocimum* samples. The first 13 samples collected were a mixture of community and commercial samples. The PCR results for these samples were highly variable (Figure 4-12) and did not sequence as well as expected (Figure 4-13). Poor sequence data could not be used to search the reference DNA database for identification of the species, or to create contigs. The few samples which had good quality sequences which could be used for identification were put into BLAST; where most of the samples were identified as being similar to one reference *O. basilicum* sequence. No suitable *O. tenuiflorum* reference sequences were found in the database.



Key:

1. Negative control
2. Positive control
3. –
4. B08
5. B07
6. B02
7. B00
8. B05
9. B01
10. B02a
11. B03
12. B04
13. B07
14. C20
15. C08

Figure 4-12 Gel Image of *matK* and *rbcL* PCR Products

The image illustrates the results of the gel electrophoresis of community and commercial tulsi samples amplified by the *matK* and *rbcL* regions. Negative control – distilled water; positive control – DNA sample known to amplify.

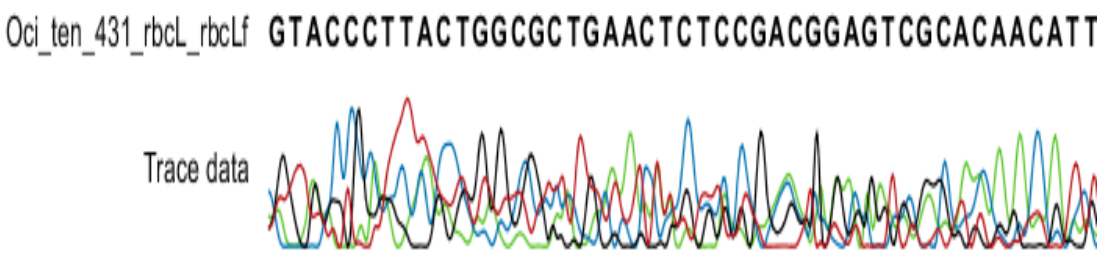


Figure 4-13 Section of the Electropherogram for the Forward *rbcL* Sequence for Sample B06g

Sample B06g represents a tulsi sample collected from participant B06 (Appendix 12).

4.6.2.3 Reference *Ocimum* Barcodes

During the initial background research it was clear that there was a lack of reliable reference barcodes to identify the tulsi samples, in the existing DNA databases (GenBank and BOLD). This meant reference DNA barcodes for the *Ocimum* species needed to be created before any identification of unknown samples could commence. Thus, authenticated *Ocimum* plants, seeds, leaves, dried material, and DNA were collected to create the reference *Ocimum* DNA sequences. During later stages of this research, in 2014, reference sequences were submitted by various sources into GenBank (Appendix 14), these were also used for the identification of unknown samples. The reference sequences created throughout this study will also be submitted to the GenBank and BOLD databases.

Creating Reference *Ocimum* barcodes

A range of authenticated *Ocimum* samples (n=33) were obtained and used to create the reference DNA sequences (Appendix 12 and 13). When a good quality DNA sequence was obtained, three reads were ordered (usually, two forward and one reverse read) to produce a reliable contig (Figure 4-14). The consensus sequence was analysed to identify any conflicts between the three sequences which needed to be resolved before it was used as the reference DNA sequence; to compare with other samples and produce a multiple alignment.

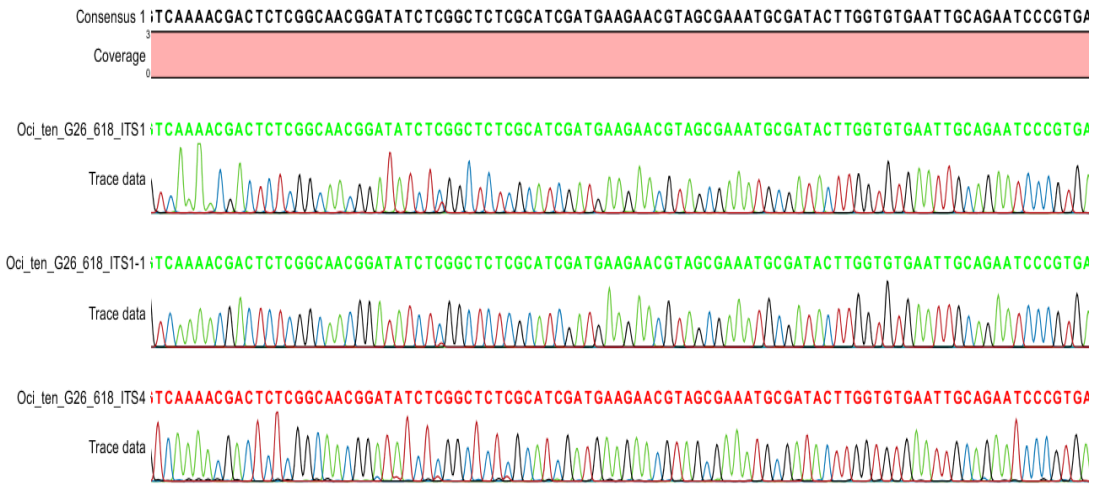


Figure 4-14 Image of a Section of a Contig Produced from Three Electropherograms of ITS Sequences from a Reference *O. tenuiflorum* Sample

The electropherograms of three ITS sequences (one reverse ITS 4 and two forward ITS 1 reads) for reference *O. tenuiflorum* sample G26 from Professor Peter Nick.

Full DNA sequences and multiple alignments can be found in Appendix 13.

For some species more than one authenticated specimen was available (i.e. from the reference specimens and DNA sequences on GenBank) and the sequences were all identical or very similar, a consensus sequence was made (Figure 4-15). Figure 4-15 shows part of a multiple alignment created with the DNA sequences of seven different authenticated *O. tenuiflorum* samples showing all the sequences were identical.

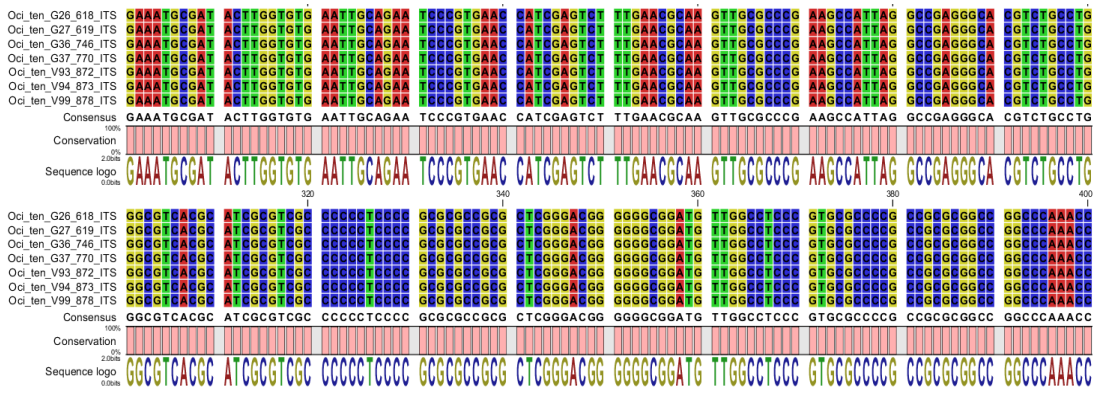


Figure 4-15 Multiple Alignment of Several Authenticated *O. tenuiflorum* ITS Sequences

The alignment of reference *O. tenuiflorum* ITS sequences represents four samples (G26, G27, G36 and G37) from Professor Peter Nick and three samples (V93, V94 and V99) from Dr. Eike Reich.

The consensus sequence from the authenticated *Ocimum* samples was then used as the reference sequence to compare unknown tulsi samples with (Figure 4-16). Figure 4-16 is a multiple alignment of several samples of which the species were unknown, with the *O. tenuiflorum* consensus reference sequence. The alignment identifies some differences between the reference and query sequences; the illustration below shows the process involved in checking whether or not the base differences are reliable.

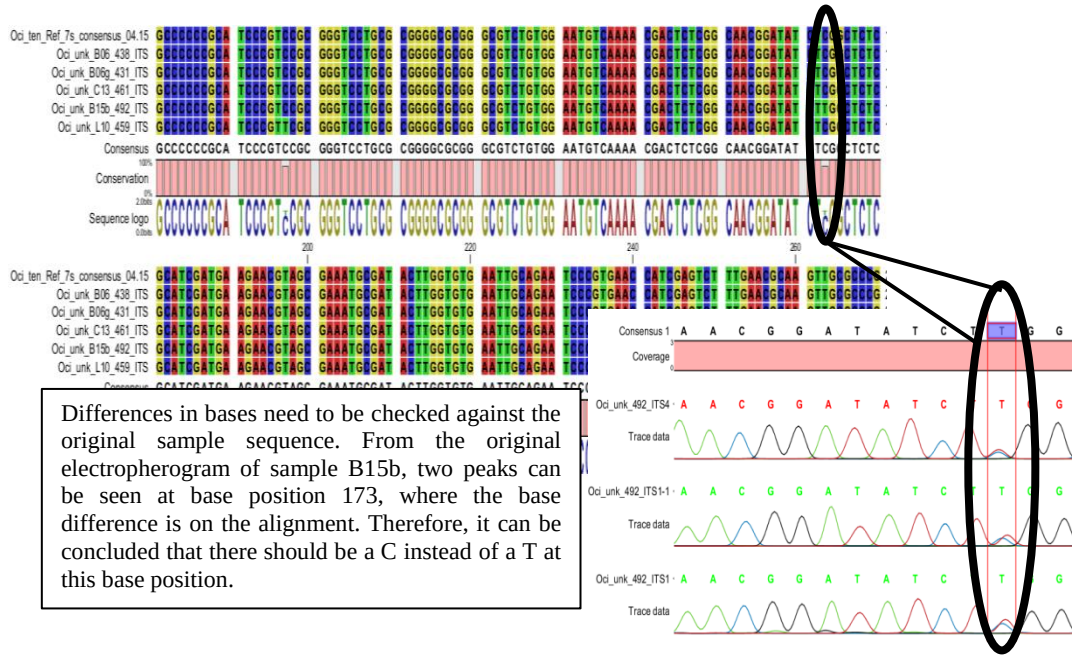


Figure 4-16 Section of a Multiple Alignment of ITS Sequences of Reference O. *tenuiflorum* Samples Used to Identify the Species of Unknown Tulsi Samples

The multiple alignment represents a reference *O. tenuiflorum* sequence (a consensus of seven different reference samples) aligned with several tulsi samples for which the species were unknown (Oci_unk).
Key: *Oci_unk_ sample identification code_extraction number_ region amplified*

From the example shown (Figure 4-16) the base difference at position 173 may be a technical error as two peaks can be seen at this position on the original electropherogram of the three sequences for sample B15b; or there may really be two different bases at this position, suggesting a polymorphism is present in the species. The quality of the electropherogram is important in helping to identify how to proceed with the analysis. In this instance the T peak seen at position 173 may be a shoulder from the previous T base continuing across the electropherogram.

ITS Sequencing of Reference *Ocimum* Specimen

Thirty three reference *Ocimum* specimens were obtained; of which 14 samples were successfully amplified with the ITS primers (ITS1 and ITS4) (Table 4-3). The sequence data for *O. tenuiflorum* and *O. gratissimum* was of high quality and the differences in the sequences can be clearly seen (Figure 4-17) along the entire sequence (546 bases), this alignment shows 75 base differences which clearly portray the differences between the two species (Appendix 13).

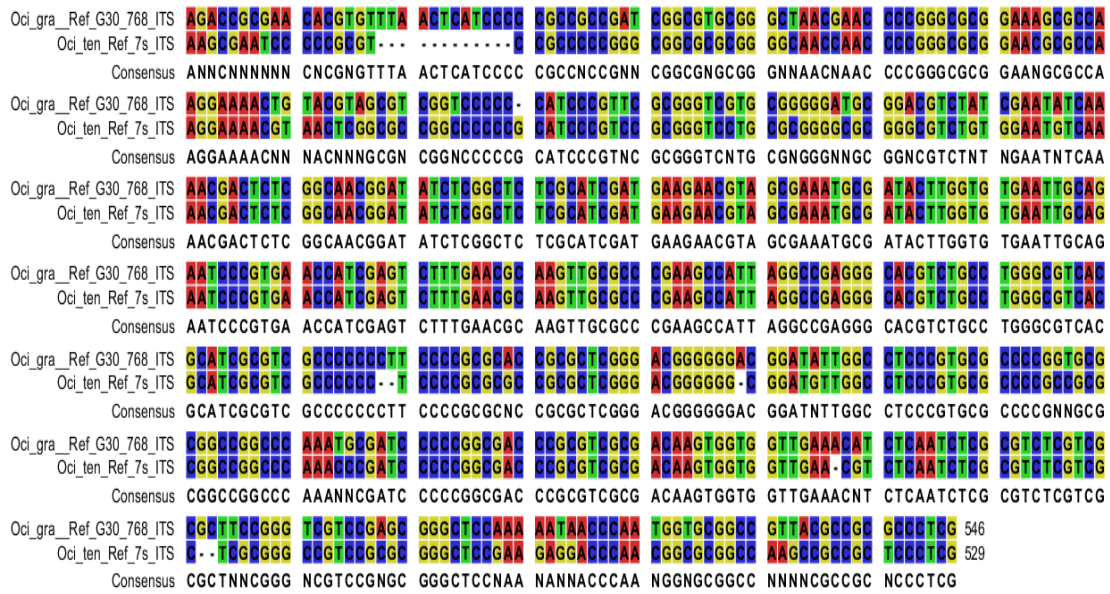


Figure 4-17 Multiple Alignment of *O. tenuiflorum* and *O. gratissimum* ITS Sequences

Oci_ten_Ref_7_ITS represents a consensus sequence of seven reference *O. tenuiflorum* sequences aligned in Figure 4-15 with Oci_gra_Ref_G30_768_ITS which represents the ITS sequence of the reference *O. gratissimum* specimen G30.

A further twelve *Ocimum* sequences were obtained from GenBank (Appendix 14) for comparison with the reference specimens collected. *O. gratissimum* and *O. selloi* sequences matched the reference sequences from GenBank. However, the reference *O. tenuiflorum*, *O.*

basilicum, and *O. americanum* sequences did not align with the sequences for the same species from GenBank.

Upon further investigation of the *O. basilicum* samples, it was noted that all four of the sequences (one reference specimen from Peter Nick and three sequences from GenBank) were very different (Figure 4-18); this could be explained by the complex nature of the ITS region, natural variance within the *Ocimum* species, or misidentification of the samples.

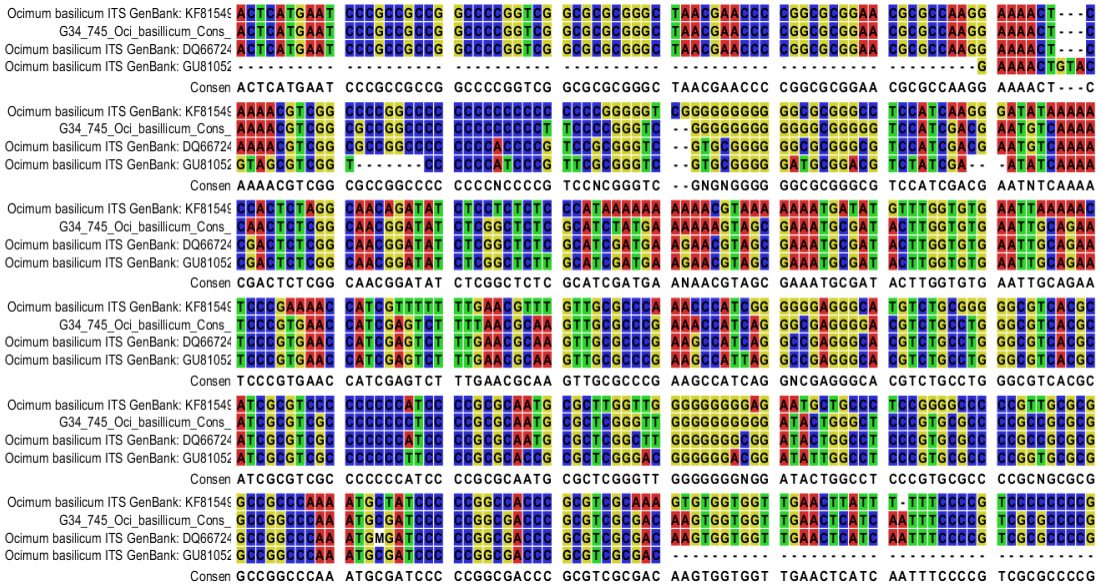


Figure 4-18 Multiple Alignment of Several *O. basilicum* ITS Sequences

The multiple alignment represents four *O. basilicum* reference sequences – three sequences from GenBank and G34 from Professor Peter Nick.

Some of the reference *Ocimum* species did not sequence as well as *O. tenuiflorum* and *O. gratissimum* with the ITS region. The sequence data was not good enough to produce reliable reference DNA barcodes (Figure 4-19). For good practice a combination of forward and reverse sequences should be used to produce a contig, the electropherograms in Figure 4-19 illustrates the problem of obtaining good quality reads in both directions.

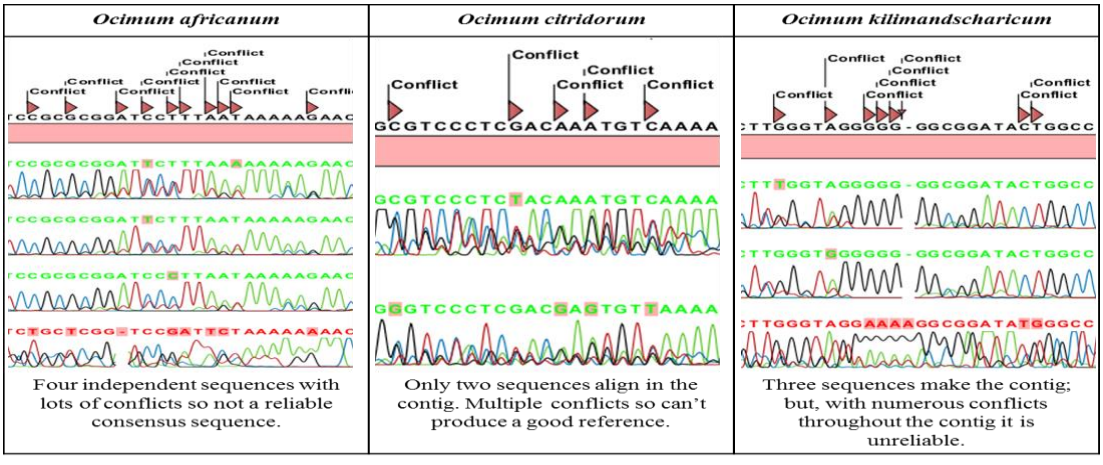


Figure 4-19 Electropherograms of Three Reference *Ocimum* Species with Poor ITS Sequencing Data

The electropherograms represent poor ITS sequences for three reference *Ocimum* species (*O. africanum, O. citridorum,* and *O. kilimandscharicum*).

trnH-psbA Sequencing of Reference *Ocimum* Specimen

Of the 33 reference *Ocimum* specimens obtained, 25 samples were successfully amplified using the *trnH-psbA* region. As the sequencing results were of high quality for most of the reference *Ocimum* specimen, reliable contigs and consensus sequences were made (Figure 4-20). Several reference *Ocimum* DNA sequences were also available on GenBank (Appendix 14). Reference specimens obtained and sequenced matched with reference DNA sequences available on GenBank, included: *O. tenuiflorum, O. gratissimum, O. basilicum, O. kilimandscharicum, O. africanum, O. americanum,* and *O. citridorum.* Therefore, these sequences were aligned with the reference consensus sequences and used to identify unknown *Ocimum* species.

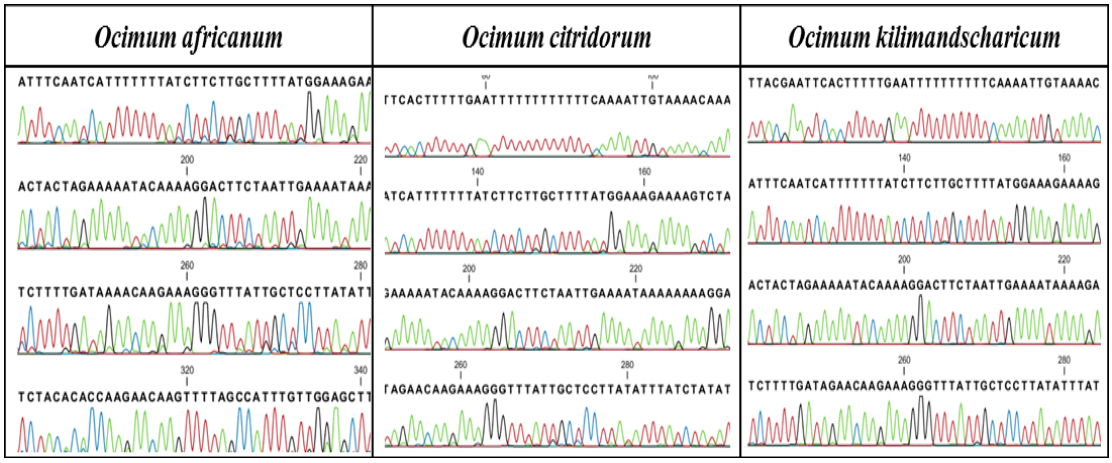


Figure 4-20 Electropherograms of *trnH-psbA* Sequences of Three Reference *Ocimum* Species

The electropherograms represent *trnH-psbA* sequences for three reference *Ocimum* species (*O. africanum, O. citridorum,* and *O. kilimandscharicum*).

Table 4-3 summarises whether or not a sequence was created for the reference specimens using the ITS and *trnH-psbA* regions. The identification of the samples was confirmed by aligning the sequences in a multiple alignment and also by entering the sequence into BLAST to compare it with other reference samples.

Once a consensus sequence was created from the reference specimens these were used to identify the species of tulsi samples collected throughout the investigation. All of the sequences were also put into BLAST to confirm the identity of the species. Samples which did not match 100% with the reference sequences created were identified by their closest match to reference sequences on the database.

Table 4-3 Reference *Ocimum* Specimens Sequencing Summary

Sample ID:	Sample source:	Type of tulsi specified:	ITS sequence:	*trnH-psbA* sequence:
G26	PN	*O. tenuiflorum*	/	/
G27	PN	*O. tenuiflorum*	/	/
G28	PN	*O. africanum*	**x**	/
G29	PN	*O. citridorum*	**x**	/
G30	PN	*O. gratissimum*	/	/
G31	PN	*O.kilimandscharicum*	**x**	/
G32	PN	*O. gratissimum*	/	/
G34	PN	*O. basilicum*	/	/
G36	PN	*O. tenuiflorum*	/	/
G37	PN	*O. tenuiflorum*	/	/
G38	PN	*O. tenuiflorum*	/	/
G39	PN	*O. tenuiflorum*	**x**	/
C81	Kew	*O. gratissimum*	/	/
C82	Kew	*O. selloi*	/	**x**
C84	Kew	*O. tenuiflorum*	**x**	/
C85	Kew	*O. americanum*	**x**	/
V86	CAMAG	*O. tenuiflorum*	**x**	/
V87	CAMAG	*O. tenuiflorum*	**x**	/
V88	CAMAG	*O. tenuiflorum*	**x**	/
V90	CAMAG	*O. tenuiflorum*	**x**	/
V92	CAMAG	*O. tenuiflorum*	**x**	/
V93	CAMAG	*O. tenuiflorum*	/	/
V94	CAMAG	*O. tenuiflorum*	/	/
V96	CAMAG	*O. tenuiflorum*	**x**	/
V98	CAMAG	*O. tenuiflorum*	/	/
V99	CAMAG	*O. tenuiflorum*	/	/

Key: PN = Professor Peter Nick (Botanical Institute Karlsruhe Institute of Technology, Germany); Kew = The Royal Botanic Gardens Kew - DNA bank; CAMAG = Dr. Eike Reich (CAMAG Laboratory, Switzerland); **x** = No DNA sequence obtained; / = DNA sequence successfully obtained.

4.6.2.3.1 *Ocimum gratissimum* Reference Specimen

Three *O. gratissimum* reference specimens were obtained: G30 and G32 from Professor Peter Nick (Botanical Institute Karlsruhe Institute of Technology, Germany), and C81 from The Royal Botanic Gardens Kew - DNA bank (Chase, M.W.). As all three samples have been identified by highly skilled botanists and identified as *O. gratissimum*, the sequence data of all three samples should align closely in a multiple alignment. Two samples G32 and C81 aligned together with no differences across 526 bases with the ITS region (Figure 4-21) and 336 bases with the *trnH-psbA* region (Figure 4-22); whereas, sample G30 did not match the other two references. The results were confirmed when the DNA samples were amplified and sequenced by both the ITS and *trnH-psbA* regions. To further validate these findings reference DNA sequences were obtained from GenBank and used in the analysis.

ITS Sequencing of *Ocimum gratissimum* Reference Specimen

Five *O. gratissimum* reference sequences obtained from GenBank were used to compare with the query samples (Figure 4-21). The alignment suggests there are two distinct varieties of *O. gratissimum* (A and B) which have not been differentiated. Across the entire sequence length ranging from 393-515 bases, for the ITS sequences, there are 14 bases which differentiate the two types of *O. gratissimum* (Appendix 13). A large number of base differences in not commonly seen in a species; but, could be explained by the high variability in the ITS region within the *Ocimum* species. To verify the difference was genuine the analysis was repeated using a different primer region (*trnH-psbA*).

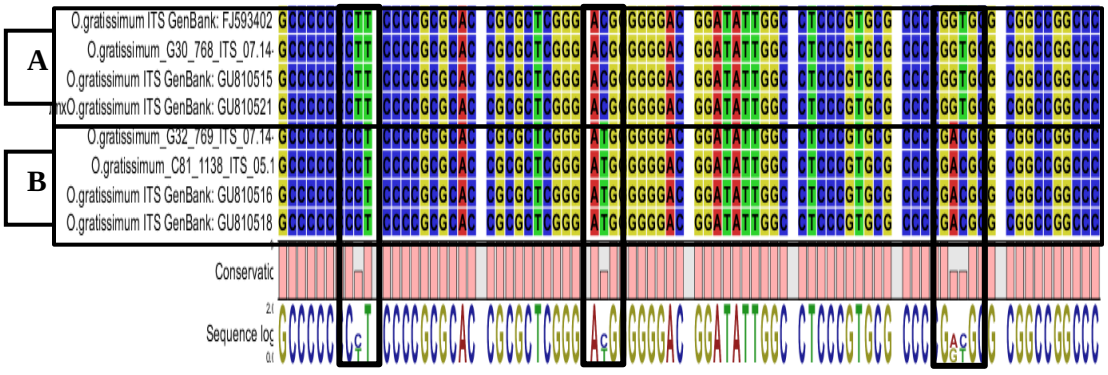


Figure 4-21 Section of a Multiple Alignment of Reference *O. gratissimum* ITS Sequences

The multiple alignment represents several reference *O. gratissimum* sequences. Samples G30 and G32 from Professor Peter Nick, C81 from Kew DNA bank and five sequences from Genbank have been used to create this alignment which identifies there are two distinct types of *O. gratissimum* (A and B).

trnH-psbA Sequencing of *Ocimum gratissimum* Reference Specimen

When searching for *trnH-psbA* reference samples, three *O. gratissimum* sequences were found on GenBank and used to compare the three reference specimens in question (G30, G32, and C81). Figure 4-22 highlights that sample G30 (A) has a perfect match with the three reference sequences from GenBank; while G32 and C81 (B) still align with each other, but are slightly different from the other four sequences. Across the 319 bases in the alignment there are just 3 base differences. Although there are not as many base differences seen with the *trnH-psbA* region, in comparison to the ITS region, the differences observed were valid. For each of the reference samples, three independent sequences were aligned in a contig to ensure a reliable consensus sequence was produced; therefore, the results from this investigation are reliable. It can be confirmed the quality of the sequence data for samples G32 and C81 was good, with clean single peaks at the base positions where the difference were found.

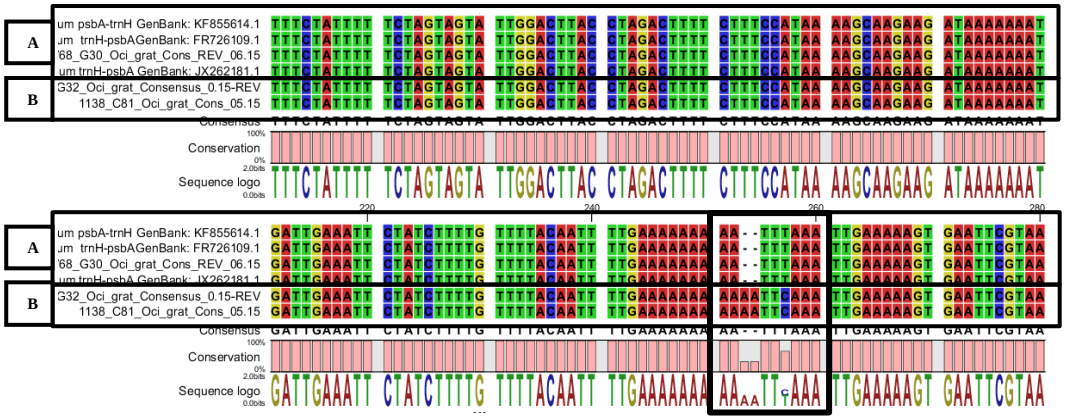


Figure 4-22 Section of a Multiple Alignment of Reference *O. gratissimum trnH-psbA* Sequences

The multiple alignment represents several reference *O. gratissimum* sequences. Samples G30 and G32 from Professor Peter Nick, C81 from Kew DNA bank and three sequences from Genbank have been used to create this alignment which identifies two *O. gratissimum* subgroups (A and B).

Summary of *Ocimum gratissimum* Results

Using both the ITS and *trnH-psbA* regions two varieties of *O. gratissimum* were identified; these were discriminated by several base differences. Although the ITS region showed greater variability between the two varieties, the *trnH-psbA* sequences were of high enough quality to support these findings. Using two regions has proved to be valuable in this investigation, as they have helped to verify the results. This study identifies the possibility of having different varieties of the same species; these may be referred to as cryptic or sibling species as they are morphologically identical but their genetic barcode may be significantly different (Sáez and Lozano, 2005).

4.6.2.4 Community Tulsi Samples

A total of 26 community tulsi samples were collected from participants encountered throughout the research period. The species for 84% (n=22) of the samples were identified using either one or both of the barcoding regions - ITS (n=15) or *trnH-psbA* (n=22). The results have been summarised in Table 4-4 which details the sample identification number, where samples were originally obtained from (donor), the donor location, reported country of origin, the type of tulsi (i.e. Raam, Shyam or unknown), and the species identification after amplification of the ITS and *trnH-psbA* regions. The different species identified have been colour coded to easily identify the species of the tulsi samples collected.

As previously highlighted there are two varieties of *O. tenuiflorum* commonly known as Raam and Shyam tulsi (Joshi et al., 2012). The results from this analysis revealed that most (n=10) of the Shyam tulsi samples collected from the UK and India were *O. tenuiflorum*; however, Raam tulsi (n=8) samples from the UK and Africa were identified as being *O. gratissimum* instead. Just one Raam tulsi sample (B102c) collected in India by participant B102 was correctly identified as *O. tenuiflorum.* One Raam tulsi sample (B04) collected from Birmingham which was a cutting passed on from a friend matched closely to *O. basilicum*. These results indicate the species of the tulsi plants are different to what they are expected to be (i.e. tulsi should be *O. tenuiflorum*). Issues with nomenclature were also identified by Jürges et al. (2009) who found that tulsi may be used to refer to any type of basil rather than a specific species. Whether or not the species of tulsi is important to people who keep tulsi is not known, but could be explored further in the future.

Of the 8 samples which were identified as *O. gratissimum*, five samples were specifically identified as being Raam tulsi by participants. Just one participant B02b thought her tulsi was a Shyam tulsi plant but instead it matched with the Raam tulsi samples identity of *O. gratissimum.* These results confirm the findings which suggest that *O. gratissimum* species may be kept as a substitute for *O. tenuiflorum* in the UK.

Three samples matched closely to *O. basilicum* reference sequences from GenBank including B04, L11 (the origins of these samples were unknown), and Li80 who noted that the sample was a basil collected from a friend‘s garden (Table 4-4). The species for four of the samples could not be authenticated. Two of these were seed samples (B12 and B105) which failed to germinate, while samples B09 and B101 failed to amplify with the ITS and *trnH-psbA* regions. Interestingly one sample (B03) from India was identified as a *Veronica* species. The seeds were germinated in a petri dish and re-grown in autoclaved soil to rule out the possibility of contamination in the soil but the same results were obtained. This could

suggest the seeds collected by this participant were not tulsi seeds and may have been wrongly identified.

Table 4-4 Community Tulsi Samples Species Identification Summary

Participant ID:	Sample ID:	Tulsi originally from (donor):	Donor Location:	Reported country of origin of tulsi:	Type of tulsi specified:	Species identification – ITS:	Species identification - *trnH-psbA:*
B01	**B01**	Friend	Birmingham	-	Unknown	*O. gratissimum*	*O. gratissimum*
B02	**B02a**	Friend	Birmingham	-	Raam	*O. gratissimum*	*O. gratissimum*
	B02b	Aunty	Leicester	-	Shyam	*O. gratissimum*	*O. gratissimum*
B03	**B03**	Mother	Amritsar	India	Unknown	*Veronica species*	*Veronica species*
B04	**B04**	Friend	Birmingham	-	Raam	*O. basilicum*[1]	*O. basilicum*[2]
B05	**B05**	Friend	Leicester	-	Raam	*O. gratissimum*	*O. gratissimum*
B06	**B06**	Friend	Leicester	-	Shyam	*O. tenuiflorum*	*O. tenuiflorum*
	B06g	Friend	Leicester	-	Shyam	*O. tenuiflorum*	*O. tenuiflorum*
B07	**B07**	-	-	-	Raam	*O. gratissimum*	*O. gratissimum*
	B07g	-	-	-	Raam	*O. gratissimum*	*O. gratissimum*
B09	**B09**	Bought from India	Punjab	India	Unknown	-	-
L10	**L10**	Cousin	India	India	Unknown	*O. tenuiflorum*	*O. tenuiflorum*
L11	**L11**	-	-	-	Unknown	*O. basilicum*[3]	*O. basilicum*[4]
B12	**B12**	Mother	India	India	Unknown	-	-
A14	**A14**	Mother-in-law	Durban, South Africa	Africa	Unknown	*O. gratissimum*	*O. gratissimum*
B15	**B15a**	Temple	Birmingham	-	Raam	*O. gratissimum*	*O. gratissimum*
	B15b	Temple	Birmingham	-	Shyam	*O. tenuiflorum*	*O. tenuiflorum*
Li79	**Li79**	Mother	Leicester	-	Unknown	-	*O. tenuiflorum*
Li80	**Li80**	Friend	Coventry	-	Basil	-	*O. basilicum*[5]
U100	**U100**	-	-	USA	Unknown	-	*O. tenuiflorum*
B101	**B101**	-	-	-	Unknown	-	-
B102	**B102a**	Mother	Gujarat	India	Shyam	-	*O. tenuiflorum*
	B102b	Mother	Gujarat	India	Shyam	-	*O. tenuiflorum*
	B102c	Mother	Gujarat	India	Raam	-	*O. tenuiflorum*
	B102d	Mother	Gujarat	India	Unknown	-	*O. tenuiflorum*
B105	**B105**	Mother	India	India	Unknown	-	-

Key: Colour of the text Blue = *O. gratissimum*; Green = *O. tenuiflorum*; Red = *O. basilicum*; Black = not identified; Grey = not an *Ocimum* species; A = Africa; B = Birmingham; L = London; Li = Leicester; U = USA.

[1] 94% match with reference *O. basilicum* sequence from Genbank (DQ667240.1)
[2] 98% match with reference *O. basilicum* sequence from Genbank (JQ339256.1)
[3] 91% match with reference *O. basilicum* sequence from Genbank (DQ667240.1)
[4] 99% match with reference *O. basilicum* sequence from Genbank (FR726106.1)
[5] 99% match with reference *O. basilicum* sequence from Genbank (FR726106.1)

Species Substitution

The two main types of tulsi recognised in traditional texts are Raam and Shyam (Krishna) tulsi which are considered to be varieties of *O. tenuiflorum* (Joshi et al., 2012). A number of tulsi samples collected from SA communities in the UK and a sample posted from South Africa were labelled as Raam tulsi (Appendix 12). Upon identification of the species using the reference DNA barcodes, it appeared that Raam tulsi samples matched with the *O. gratissimum* reference sequences instead of *O. tenuiflorum.* This suggests that the species of Raam tulsi plants has been confused and substitution has occurred. Migration of SA communities from India to Africa and then to the UK may have affected the transfer of seeds and caused confusion of species. *O. gratissimum* is native to Africa and known to grow abundantly even in the wild; whereas, it is difficult to find in India and does not grow as well. Due to similar morphological features, it may have been misidentified by people or kept as a substitute as the preferred type was not available. Findings from the interviews for this research suggest Raam tulsi was easier to germinate and grow in comparison to Shyam tulsi, in the UK. The results suggest species substitution has occurred for Raam tulsi which appears to be *O. gratissimum* instead of *O. tenuiflorum.*

Although reference specimens G32 and C81 were identified as *O. gratissimum* they appeared to be different to reference G30 (Figure 4-23), which aligned with all the community tulsi samples identified as Raam tulsi. From this analysis it can be concluded that two varieties of *O. gratissimum* were identified.

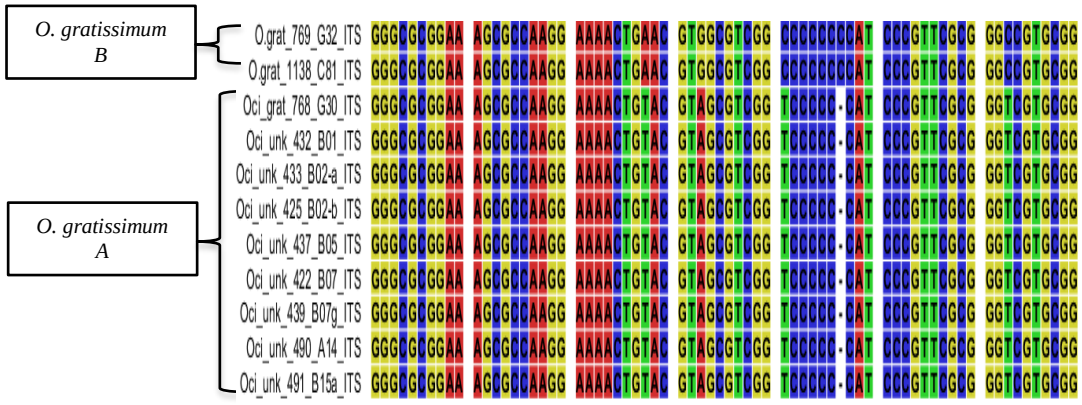


Figure 4-23 Section of a Multiple Alignment of ITS Sequences of Unknown Tulsi Samples with Authenticated *O. gratissimum* Samples A and B

The multiple alignment represents the two types of reference *O. gratissimum* sequences (A and B) aligned with several community tulsi samples for which the species were unknown (Oci_unk).
Key: *Oci_unk_extraction number_sample identification code_region amplified*

Two community tulsi samples (B04 and L11) matched closely to *O. basilicum* reference sequences instead of *O. tenuiflorum*. This further supports the results which suggest the species of tulsi plants kept in the UK may have been substituted by alternative *Ocimum* species.

4.6.2.5 Commercial Tulsi Samples

A range of commercial products were brought from around the UK, India, and online. In total 47 samples including: capsules, creams, soaps, syrups, shampoos, teas, tablets, oils, toothpaste, and a tulsi rosary were bought. Raw tulsi material used by Pukka Herbs was also included in this collection. Table 4-5 summarises the species identification for the samples which were identified using the ITS and *trnH-psbA* regions. Just 5 commercial samples were identified using ITS primers (ITS 1 and ITS 4), while 13 samples were identified with the *trnH-psbA* region (complete list of the commercial products used in this analysis can be found in Appendix 12).

Table 4-5 Commercial Tulsi Samples Species Identification Summary

Sample ID:	Sample source:	Type/ Species of tulsi specified:	Type of Sample used for DNA extraction:	Species identification – ITS:	Species identification - *trnH-psbA:*
B00	Basil plant from Tesco	*O. basilicum*	Fresh leaves	*O. basilicum*	*O. basilicum*
C08	Pukka three tulsi tea bag	Species not specified. *Raam, Shyam & lemon tulsi*	Dried leaf material from inside a tea bag	-	*O. tenuiflorum*
C13	Tulsi seeds from ebay	*O. tenuiflorum (*Shyam*)*	Seeds – germinated. DNA extraction from leaves	*O. tenuiflorum*	*O. tenuiflorum*
C16	Akamba garden centre	*O. basilicum*	Fresh leaves	*O. basilicum*	*O. basilicum*
I44	Market in Fort Kochi, India	-	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
I45	Phalada Agro farm	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
I46	Phalada Agro farm	*O. tenuiflorum*	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
I47	Phalada Agro farm	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
I48	Phalada Agro farm	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
C73	Pukka Herbs	Species not specified. *(Raam)*	Dried leaves	-	*O. tenuiflorum*
C74	Pukka Herbs	Species not specified. *(Raam)*	Dried leaves	-	*O. tenuiflorum*
C76	Pukka Herbs	Species not specified. *(*Shyam*)*	Dried leaves	-	*O. tenuiflorum*
C78	Pukka Herbs	Species not specified. *(lemon Vana)*	Dried leaves	-	*O. basilicum*

Despite the samples being subjected to various procedures to try and produce a positive PCR result (e.g. a new extraction of the sample, an isopropanol clean up, or diluting the DNA) the

species for many (n=34) of the commercial products could not be identified. Jürges et al. (2009) also experienced similar problems of extracting tulsi DNA from multicomponent formulations. The *trnH-psbA* region was able to amplify more commercial samples in comparison to the ITS region. One of the reasons why the *trnH-psbA* region might have produced better PCR results for the commercial samples is because it is a shorter fragment; therefore, making it easier to amplify samples which may have been degraded during the manufacturing processes.

4.6.2.6 The nuclear ITS Region

The use of the ITS region for producing DNA barcodes to identify various species has been investigated with varying degrees of success (Jürges et al., 2009; Selvaraj et al., 2012); yet little is known about its suitability as a barcode for the *Ocimum* species. This investigation explored the suitability of the ITS region as a barcode to differentiate between *Ocimum* species. As little documented data is available, the results from this study can be used to contribute to the gap in the literature.

In this investigation the ITS region provided variable quality sequencing data. Of the total 111 samples collected, two samples were excluded from the analysis as they were seeds which failed to germinate and DNA was not successfully extracted. A further 27 commercial samples including: capsules, tablets, shampoos, creams, and soaps were also excluded after several attempts to amplify the samples with no success. Hence, 82 samples were included in this analysis, of which the ITS region was used to successfully identify 35 (43%) samples (Appendix 12); this included the identification of 14 community, 5 commercial, 14 reference, and 1 miscellaneous sample.

The ITS region shows a high level of discriminatory power for some species (Li et al., 2011); this was observed for the *O. tenuiflorum* and *O. gratissimum* samples analysed. The ITS region is known to be better at discriminating species than some of the plastid regions; however, the limitations associated with the ITS region have a key in influencing the use of these primers (Hollingsworth et al., 2011). As identified by Kress et al. (2005) the reason the ITS region may not sequence as well in some plants is due to the problem of paralogy and issues associated with this highly repeated part of the nuclear genome.

It was observed that samples which were either *O. tenuiflorum* or *O. gratissimum* were often straight forward to PCR and amplified well; in addition, they produced good quality sequencing data for analysis. The PCR worked better on fresh and dried plant material as opposed to samples which may have been exposed to manufacturing processes (e.g.

capsules, tablets, and tea bags). For commercial products and other *Ocimum* species (e.g. *O. africanum, O. basilicum, O. citridorum,* and *O. kilimandscharicum*) the PCR results were not as easily obtained. Samples which failed to produce a single bright band on the gel were then subjected to various processes. For example, the PCR was repeated using a lower concentration of DNA by diluting the sample (1 in 10), or an isopropanol clean-up of the DNA sample was carried out to remove any inhibitors in the sample which maybe inhibiting the PCR reaction. Of the 35 samples which gave a positive PCR result with the ITS region, 54% gave a clear PCR result and a good quality sequence on the first attempt; all the others required further optimisation before good quality sequencing data was obtained. Although several samples gave a positive PCR reaction result the data could not be used for the analysis as the quality of the sequencing data was poor.

The results from this investigation show that the ITS region could be a potential barcode for authenticating some of the *Ocimum* species (e.g. *O. tenuiflorum* and *O. gratissimum*). But not all of the *Ocimum* species were successfully amplified; thus, the use of other barcode regions should also be explored.

Problems Encountered with the ITS Region

Several challenges were encountered while working with the ITS region including: difficulty amplifying and sequencing the region, poor quality sequencing data, and fungal contamination.

❖ Multiple Bands

Several samples displayed multiple bands on the gel electrophoresis (Figure 4-24). This may indicate the presence of more than one species in the DNA sample; i.e. multiple species if a mixed sample is used, or contamination. The DNA for these samples was extracted from a single plant so there was no chance of the sample being mixed. A DNA ladder was used to estimate the size and quantity of the DNA fragments. In Figure 4-24, the fragments look very similar;

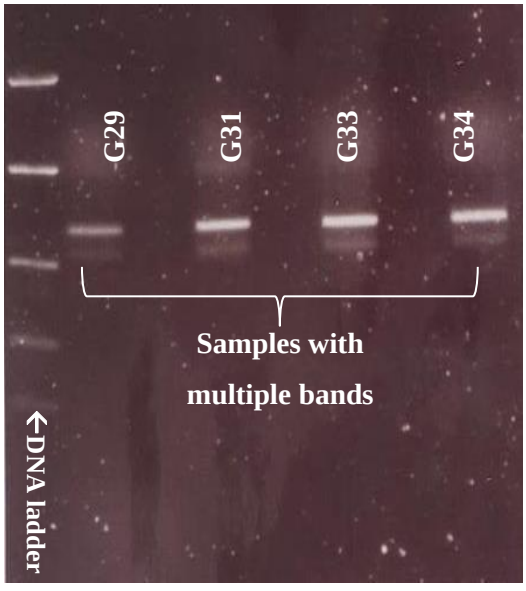


Figure 4-24 Gel Image of *Ocimum* Samples with Multiple Bands

this was investigated further by extracting the DNA from the individual bands, repeating the PCR to amplify the DNA, and then sequencing the samples. The DNA sequencing of the individual bands revealed that they had the same sequences. This could be due to the formation of secondary structures (Kress et al., 2005).

❖ **Poor ITS Sequencing Data**

Some of the *Ocimum* species did not sequence as well as *O. tenuiflorum* and *O. gratissimum* with the ITS region. The messy DNA sequences may be due to paralogous gene copies; the multiple copies of the ITS region within cells can lead to different variants being simultaneously sequenced (Hollingsworth, 2011). This problem can cause the same species to be given several different identities depending on different laboratory protocols, and chance (Hollingsworth et al., 2011). Poor quality sequencing data may also be caused by the formation of secondary structures by the ITS primer which can interfere with the sequencing (Kress et al., 2005). In addition, the GC content can also be a factor which can affect the quality of the sequence. The GC content is the fraction of Guanine (G) and Cytosine (C) nucleotides in the amplicon. GC rich regions can contribute to the formation of secondary structures, and often require optimisation (e.g. higher annealing temperatures) before suitable amplification can occur (Henke et al., 1997), making sequencing very difficult (Fazekas et al., 2010). A high GC content can increase the possibility of mis-priming; thus affecting the quality of the sequencing date. The GC content for the *Ocimum* samples varied between 45-72%. Some samples with a high GC content did have poor quality sequence data in comparison to those with a lower GC content.

❖ **Amplification of Fungal Contamination**

The sequencing results for several samples revealed fungal DNA had been amplified instead of the plant DNA. This is a well-known problem with the ITS region, as the fungal ITS region is so similar to that in plants it can be unintentionally amplified (Hollingsworth et al., 2011). Various stages were repeated; for example, a new DNA extraction, an isopropanol clean-up of the original DNA sample, another PCR with the original/cleaned/diluted DNA sample. Some of the samples eventually gave a plant species; but, some samples still appeared to amplify the fungal contamination rather than the plant DNA. For example, samples I45 and I49 collected in India were identified as *Alternaria alternate* (Fr.) Keissl. The results for these samples could not be used.

4.6.2.6.1 Application of ITS2

It has been suggested that if the full ITS region is difficult to sequence a portion of it (ITS2) may provide a useful alternative (Li et al., 2011). As the ITS2 region is a smaller fragment of the ITS region it can reduce the amplification and sequencing problems associated with the entire ITS region (Kress et al., 2005; Hollingsworth et al., 2011). Samples which did not give good quality results with the full ITS region (ITS1 and ITS4) were then sequenced with the ITS2 primer. The ITS2 primer is kept as a universal primer by Macrogen, hence several samples were re-sequenced with the ITS2 primer in addition to ITS1 and ITS4 using the Macrogen online ordering facility. However, the results were still not as good as expected as the sequencing data was poor and could not be used for the analysis.

4.6.2.7 The plastid *trnH-psbA* Region

The results from this analysis confirm the high level of interspecific variation amongst the *trnH-psbA* region in the *Ocimum* genus. The region amplified and sequenced samples efficiently and produced good quality sequence data. PCR with the *trnH-psbA* primers was conducted with the 82 *Ocimum* samples (community, commercial, and reference); of which the species for 66 samples (80%) were identified including: 21 community, 13 commercial, 25 reference, and 6 miscellaneous samples. Reactions were conducted personally and by a Graduate Champion Research Student (Linnette Sleigh) under supervision. The initial PCR and gel electrophoresis results appeared to work more efficiently without the need to clean the DNA or repeat the PCR, unlike with the ITS region. In general, most of the samples gave good quality sequencing data on the first attempt.

Some species may have low variability, making it difficult to distinguish between species (Newmaster et al., 2007). This was observed with the two types of *O. gratissimum* (A and B), where only 3 base differences were identified by *trnH-psbA*, over 334 bases; whereas, the ITS region identified 21 base differences across 526 bases. If specific primers to distinguish between the two types of *O. gratissimum* species were to be created using the *trnH-psbA* results it would be more difficult, because of how similar the sequences are.

Similar to results published by Christina and Annamalai (2014) and the British Pharmacopoeia (2016), the *trnH-psbA* region proved to be useful for authenticating the *Ocimum* species in this investigation.

4.6.3 Scanning Electron Microscopy Results

Several of the reference *Ocimum* specimens obtained from Professor Peter Nick (Figure 4-25) and some community tulsi samples were analysed using SEM (Figure 4-26), to confirm

the identity of the samples. The results revealed the unique differences in the surface structures of the samples analysed. The *Ocimum* species are all aromatic plants; hence, oil glands which secrete the aromatic oils can be found dispersed across all of the leaves (Maimes, 2004). The size and number of oil glands are characteristically different in the *Ocimum* species, and can be used to distinguish between the samples (Suddee et al., 2005).

Differences in hair structure and density were identified. *O. africanum, O. basilicum* and *O. citridorum* are described as having hairless leaves (i.e. there are no trichomes on the surface of the leaves) (Paton, 2014); this is evident in Figure 4-25 as the surface of the leaves are glabrous. On the other hand, *O. gratissimum* and O. *tenuiflorum* do have hairs on the upper and lower epidermis of the leaves. Differences between the two species can be seen; *O. gratissimum* has a higher density of trichomes covering the entire surface of the leaf in comparison to the *O. tenuiflorum* sample. Although *O. tenuiflorum* does have trichomes, there are fewer of them and they are widely dispersed around the leaf.

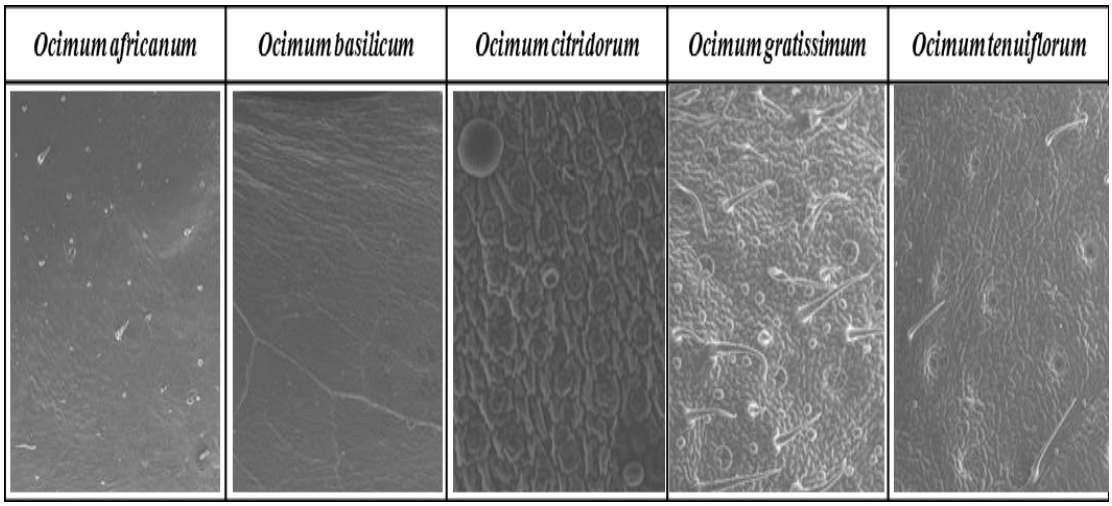


Figure 4-25 SEM Image of *Ocimum* Species
SEM images of the lower epidermis of five different *Ocimum* species (*O. africanum, O. basilicum, O. citridorum, O. gratissimum,* and *O. tenuiflorum*) taken at De Montfort University by R. Armitage and S. Bhamra.

Figure 4-26 shows the differences in surface structures between fresh and dried Raam and Shyam samples, collected from participants in the UK. Both tulsi samples have numerous oil glands, which are classical characteristics of the Lamiaceae family. However, the Raam tulsi sample has more trichomes covering the leaf in comparison to the Shyam tulsi leaf. These morphological differences can help distinguish between different species. These SEM images confirm the DNA analysis results which found Shyam tulsi was *O. tenuiflorum* while the Raam tulsi samples was *O. gratissimum.*

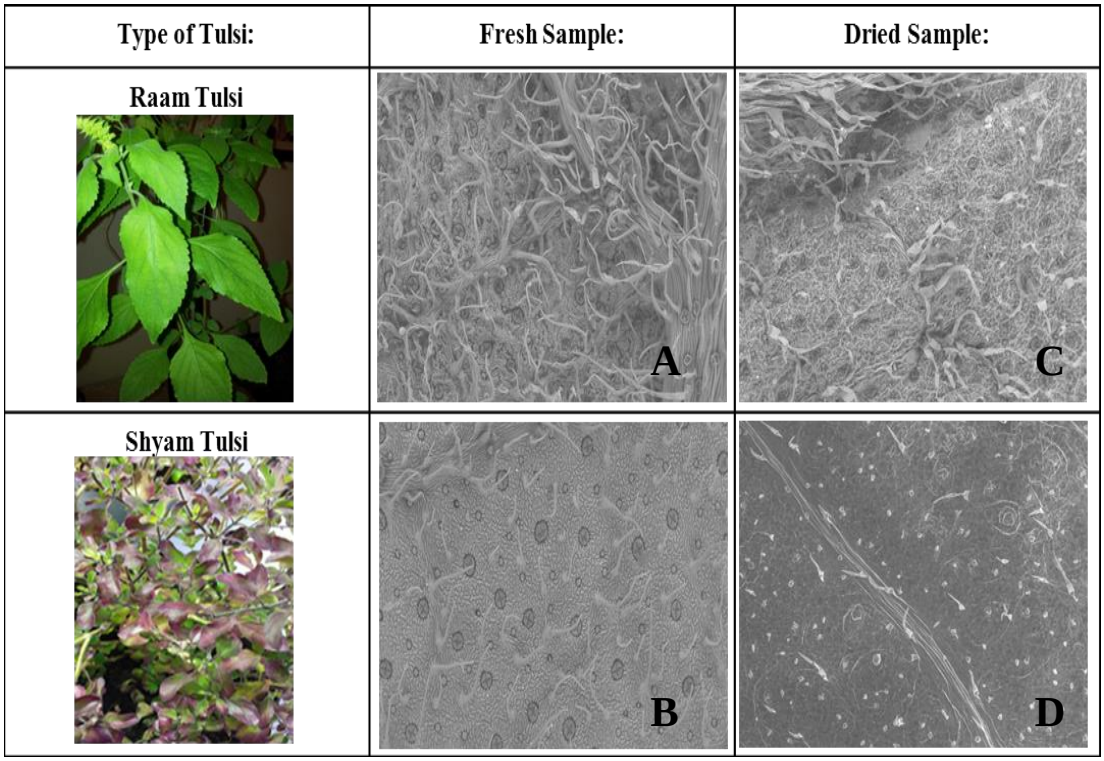


Figure 4-26 SEM Comparisons of Raam and Shyam Community Tulsi Samples Collected in the UK

A = lower epidermis of a fresh Raam tulsi leaf; B = lower epidermis of a fresh Shyam tulsi leaf; C = lower epidermis of a dried Raam tulsi leaf; D = lower epidermis of a dried Shyam tulsi leaf, taken at De Montfort University by R. Armitage and S. Bhamra.

SEM can provide valuable information about the surface structures of different species, which can help differentiate between them. All of the images in Figure 4-25 and Figure 4-26 A + B used fresh leaf samples for the SEM; the surface structures were clear and easier to identify in these images. Dried leaf material imaged by SEM (Figure 4-26 C + D) was difficult to analyse as the structures had dehydrated it was more difficult to identify them. Jacono (2009) observed that when plant material was dried or cut into smaller fragments it became difficult to identify the plant from its morphology; this was observed with the dried samples used in this investigation. Jacono (2009) claims the most reliable way to identify a plant is while it is fresh, either alive in its habitat or having been recently collected; although, adulterants may sometimes resemble the same characteristics as a genuine sample (Joshi et al., 2004). This illustrates the importance of having highly skilled professionals involved in the identification of plants and the use of additional authentication techniques to verify the identification of the species.

4.7 Tulsi Project Summary

4.7.1 The Current Use of Tulsi by South Asian Communities in the UK

The interview results revealed what tulsi plants were being used for, where tulsi plants, products, and seeds were sourced from, and what value the plants had amongst SA communities in the UK. In total 13 interviews were conducted which enabled in-depth data to be gathered and analysed. Most of the interviews were conducted face-to-face by the researcher, although several were done via telephone due to the location. Interviews with participants from across Birmingham, Leicester, and London were conducted, with the collection of tulsi samples for molecular analysis.

Two main varieties of tulsi were identified and kept by participants; which were Raam and Shyam (Krishna) tulsi. Raam tulsi was described as having large flat, bright green leaves which often grew quite tall; whereas, Shyam tulsi had distinctly smaller, darker green to purple leaves, and was harder to grow. Of the population surveyed 92% of participants (n=12) claimed to grow their own tulsi plants, with varying degrees of success in the UK. Reference to how the cold and damp climate in the UK was not ideal for the growth and survival of tulsi was noted. Participants who grew their plants from seeds revealed that they had got their seeds from family, friends, and religious institutes; both in the UK and abroad - from Africa or India. The sharing of seeds and cuttings was common practice amongst the SA communities. The different species of tulsi grown and used in the UK may be explained by the different migration and settlement patterns of SA communities.

The knowledge of what the plants were used for and how they should be maintained was passed on predominantly by older members of the family (mothers and grandparents), and priests and scriptures from the Hindu religion. Due to the representation of the Hindu goddess Virinda Tulsi, it was evident that the tulsi plants had a prestigious position within Hindu communities. It was observed that the plants were cared for and kept as religious figures in specific locations either in the front or centre of the home, and in temples (*mandirs*). Participants believed it was a blessing to be able to keep a tulsi plant in the home. The results identified numerous medicinal benefits of tulsi including the use in treating sore throat, cough, diabetes, asthma, and skin conditions.

The findings of this research have explored the current position of the tulsi plant amongst SA diasporic communities, in the UK. The range of medicinal, culinary, cultural and religious uses of tulsi makes this plant highly valuable. The vast array of commercial products available on the market are designed to be easy and convenient for people to utilise the health benefits of the plant around the world, even if they do not have access to the plant.

4.7.2 Identification of Plant Species

Traditional methods of plant identification involve the use of morphological features, where botanists have looked for particular traits to identify plant families and species. Techniques such as Scanning Electron Microscopy (SEM) have been improved to be able to distinguish microscopic plant features, without destroying the surface structures (Pathan et al., 2008). The SEM images captured during this research were able to illustrate the differences between the species; just as Dr Alan Paton had suggested (Paton, 2014). The *O. gratissimum* sample had significantly more trichomes (hair like structures) on the leaf surface in comparison to the other *Ocimum* species; while the smooth, glabrous surface of the *O. basilicum* leaf was seen clearly in the SEM images. Although, when samples were dried it was difficult for the lay person to distinguish between the different species; with the correct training the results could be better interpreted and understood. SEM images were only taken of some of the reference specimens (i.e. *O. africanum, O. basilicum, O. citridorum, O. gratissimum, and O. tenuiflorum*) to explore the differences in surface structures between the different species. The results from the SEM analysis were able to support the molecular results; i.e. the different surface structures where characteristic of the different species.

The age of the plant, condition of the sample and manufacturing process can affect the characteristics of the samples, making them difficult to identify without the correct knowledge (Jacono, 2009). Likewise, chemical analysis is also affected by the natural variance in plants, growing conditions, processing, and storage (McCutcheon, 2002). This is where DNA analysis provides its unique advantages as it is not affected by the age of the plant, condition (e.g. fresh or dried), growth conditions, or manufacturing processes (e.g. drying and storage). It is a relatively new technique for plant authentication which requires more research, in comparison to the other identification methods which have a long standing and reputable use. The results from this investigation have revealed the usefulness of DNA analysis and can be used to contribute to this evolving discipline.

4.7.3 The Applications of DNA Barcoding for Medicinal Plant Identification

While conducting background research into *Ocimum* DNA sequences it became apparent that there was a lack of reliable reference *Ocimum* DNA sequences available in the databases such as GenBank and BOLD. Chase et al. (2005) highlighted this as a potential limitation of DNA authentication, as there is a lack of DNA barcodes available in the reference databases. Before authentication of tulsi samples could commence a reference database needed to be created to be able to verify the species. This research has had an important role in collecting

a range of authenticated *Ocimum* specimens (n=33) from around the world to create reference barcodes which will make future authentication faster and reliable; in addition, these sequences will be submitted to the GenBank and BOLD databases.

As recommended by The Consortium for the Barcode of Life (CBOL) Plant Working Group, initially the plastid *matK* and *rbcL* regions were explored for amplification of DNA samples (Kress et al., 2005; Chase et al., 2005). The PCR results were highly variable and the sequence data for the few samples which did provide a positive PCR result were very poor. From the literature it was clear these regions are highly valuable; however, their use with the *Ocimum* species was limited and required optimisation. When other regions such as the nuclear ITS and plastid *trnH-psbA* were explored amplification and sequencing results were much better. The ITS region had limited success as it was only able to amplify and sequence some of the *Ocimum* species (*O. tenuiflorum, O. selloi, and O. gratissimum*), while the *trnH-psbA* region provided better DNA sequencing data for a larger range of species. Of the total 111 tulsi and *Ocimum* samples collected during the research period, 82 samples were used for the final analysis of which 67 samples (82%) were successfully identified with the combination of the ITS and *trnH-psbA* regions.

In order for DNA barcoding to work there must be enough variation in the DNA sequence to differentiate species from one another (interspecific variation); and a much lower level of variation within a species (intraspecific variation) (Lahaye et al., 2008). The intraspecific variation is known to be relatively low for both ITS and *trnH-psbA* (Kress et al., 2005). The results from this investigation revealed that the ITS region had higher intraspecific variation than the *trnH-psbA* region, as it was able to identify more base differences in the DNA sequence. The ITS region was highly variable but limited due to poor quality sequence data. The *trnH-psbA* region provides a high level of interspecific variation, therefore allowing the discrimination of different species (Kress et al., 2005). The results from this study revealed that both the ITS and *trnH-psbA* regions were able to distinguish and identify several *Ocimum* species; nevertheless, more samples (n=66) were identified using the *trnH-psbA* region than the ITS region (n=35).

Several challenges of using the ITS region were encountered during the investigation, including the difficulty amplifying and sequencing samples, the amplification of fungal DNA instead of the plants, and poor quality sequence data possibly due to paralogous copies of ITS region being present. In cases where the complete ITS region was difficult to amplify and sequence the ITS2 region was recommended and tried; but, no improvement in results were obtained. The *trnH-psbA* region worked more efficiently than the ITS region and provided higher quality sequence data. Although, there is a risk of low variability within

species, the differences found for the *Ocimum* species was sufficient to discriminate between them. This was also observed by Hollingsworth (2011) who claimed the *trnH-psbA* region had better discriminatory power, and gave more efficient recovery of sequence data compared to other primer regions such as *matK* and *rbcL*.

It has been proposed that a combination of regions should be used for the authentication of species (Kress et al., 2005; Li et al., 2011); this was successfully completed in this study and the results validated each other. A multigene approach using different priming regions such as the nuclear ribosomal ITS and plastid *trnH-psbA* regions have been proposed as combinations for DNA barcoding (Chase et al., 2005; Kress et al., 2005). This study has implemented these proposals and discovered some interesting results. Both regions have had varying degrees of success with the *Ocimum* species. The *trnH-psbA* region proved to be better than the ITS region at sequencing the *Ocimum* samples, as it was able to authenticate more than double the number of samples.

Reference specimens (n=33) obtained from several sources (Professor Peter Nick - Botanical Institute Karlsruhe Institute of Technology, Germany; Dr. Eike Reich - Director at CAMAG Laboratory, Switzerland; and The Royal Botanic Gardens Kew - DNA bank) were used to create reference DNA sequences to identify the species of samples collected throughout the project. A large proportion of the samples collected for analysis (n=67, 82%) were identified using the reference DNA barcodes created. Fresh and dried leaf samples were the easiest to extract DNA from, amplify and sequence. A total of 26 community samples were collected of which 81% (n=21) were identified as an *Ocimum* species. Commercial products which had been processed were often difficult to extract DNA from, as identified by the NanoDrop analysis which often showed little or no absorbance at 280nm. This may be due to manufacturing processes involved in formulating the products which may have damaged and degraded the DNA. Although one of the advantages of DNA analysis is that it is not affected by manufacturing processes, the results from this study found that steam treatment and drying in extreme temperatures did degrade the DNA of commercial samples collected (n=47); making it very difficult and sometimes impossible to extract DNA. But, because the tr*nH-psbA* region is a shorter fragment it worked better with commercial samples (i.e. 13 commercial samples species were identified) where the DNA may have been degraded or sheared into smaller fragments, in comparison to a longer region such as the ITS (i.e. 5 commercial samples species were identified). A large number of the commercial samples (72%, n=34) could not be used in the analysis as DNA was not successfully extracted, similar problems were also encountered by Jürges et al. (2009) who also struggled to authenticate the species of commercial tulsi products.

4.7.4 The Key Discoveries of the Tulsi Project

The cultural and commercial values associated with the tulsi plant can increase the risk of adulteration due to the constant and rising demand of the market (Christina and Annamalai, 2014). The correct identification of tulsi plants is made difficult by the similar morphological features of the *Ocimum* species. Raam and Shyam tulsi samples collected in India looked very similar; whereas, samples from the UK were distinctly different. Both Raam and Shyam tulsi are supposed to be varieties of *O. tenuiflorum*; however, the DNA analysis identified Raam tulsi samples collected from SA communities in the UK and Africa as *O. gratissimum* instead. According to the results from this investigation, Raam tulsi species have been substituted in the UK; but how relevant this is to SA communities remains to be investigated. Many of the commercial samples collected did not specify the type of tulsi (i.e. Raam or Shyam), except for samples from Pukka Herbs®; the Raam and Shyam tulsi samples from Pukka Herbs® did match the *O. tenuiflorum* references. In addition, Raam and Shyam samples collected personally during field work in India were also correctly identified as *O. tenuiflorum;* further supporting the theory of species substitution in the UK. Plant species which are difficult to source, expensive, and have limited availability are subject to adulteration with products which maybe morphologically similar. Using taxonomically correct plants is critical to the safety and efficacy of herbal medicine (WHO, 2002b). Adulteration of plants can have fatal consequences; therefore, the importance of selecting the correct plant species is of paramount importance, especially when consumed for medicinal purposes (Christina and Annamalai, 2014).

The main reasons for keeping tulsi plants identified by participants in the UK were for cultural or religious purposes, in which case the species of the plant may not be as important. Different species may have different pharmacological effects; therefore, the species used for commercial and medicinal purposes must be properly identified and authenticated. DNA analysis has proved to be a technique which can accurately identify and discriminate between the *Ocimum* species.

The results of this investigation have enabled new reliable reference DNA sequences for the *Ocimum* species to be created and will contribute to the DNA databases currently lacking this information (Appendix 13 & 15). This will enable more efficient authentication of *Ocimum* samples to take place. The ITS and *trnH-psbA* regions have been able to successfully authenticate and discriminate several *Ocimum* species. A range of unknown *Ocimum* samples have been identified, illustrating the usefulness of this technique.

5.0 Chapter 5 - Discussion

5.1 Overview of the Research Outcomes

The research set out to explore the knowledge and use of traditional herbal remedies amongst South Asian (SA) diasporic communities and healthcare professionals (HCPs) in the UK. The results of this investigation have provided an insight in to the current use of herbal medicines (HMs) by South Asians (SAs), where ingredients for HMs are sourced from, how minor ailments are treated, and where the knowledge of HMs originates from and is transmitted through generations (Chapter 2). Furthermore, patients' perspectives of HMs (i.e. SA participants taking conventional Western medicines) were compared with the views of HCPs in the UK (Chapter 3). SA participants and HCPs views of HMs and some of the key finding from this research have been summarised in Figure 5-1. Figure 5-1 highlights how the results of these two phases of the research identified the problem of species substitution and the need for authentication of HMs; as well as identifying a medicinal plant of interest (Tulsi, *Ocimum tenuiflorum* L.) for DNA analysis. Tulsi was one of the most popular medicinal plants mentioned by participants in both strands of the research; the religious significance and numerous medicinal benefits associated with the plant make it a culturally and commercially valuable plant.

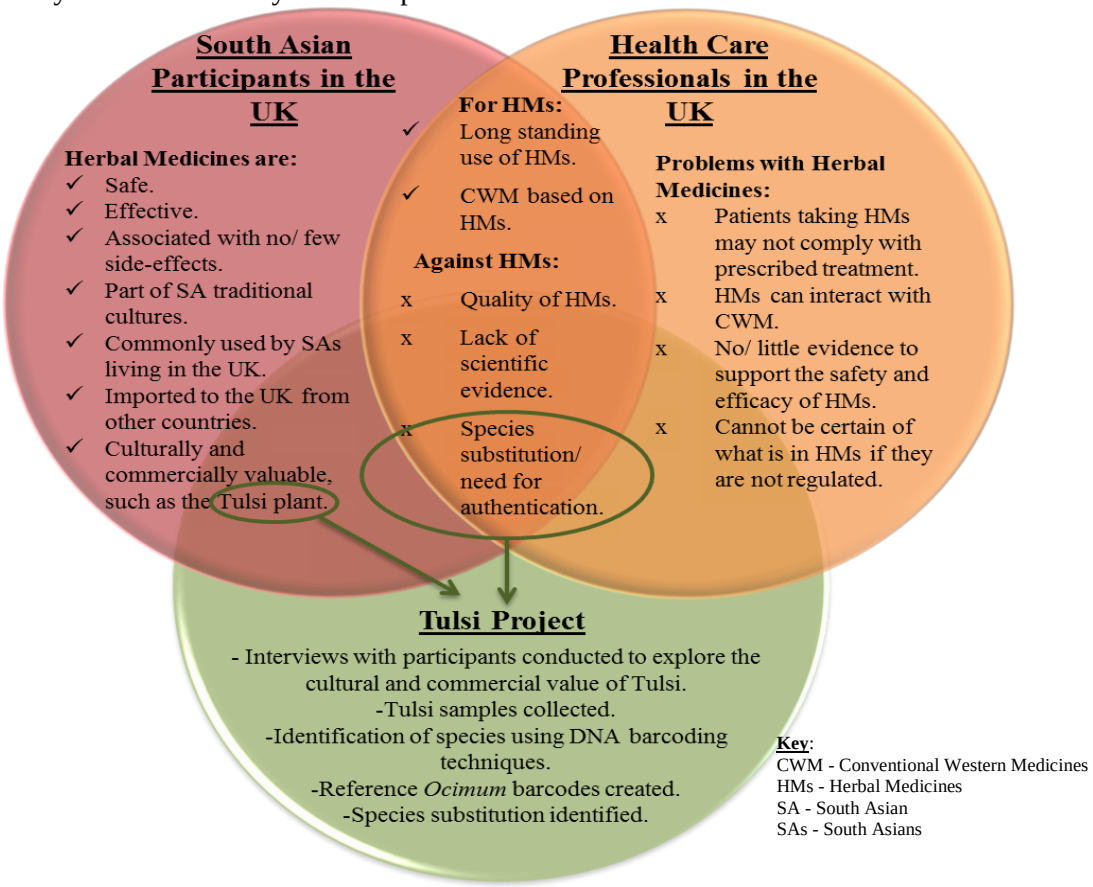


Figure 5-1 Venn Diagram of South Asian Participants and Healthcare Professionals views of Herbal Medicines and the Connection of the Three Phases of the Study

The Venn diagram summarises some of the key themes identified and illustrates how the three phases of the research (i.e. SATMED Project, HCPs survey, and the Tulsi Project) interlink and led on to the identification of a medicinal plant of interest (i.e. Tulsi) for DNA analysis.

The use of DNA barcoding in ethnobotany is relatively new (Newmaster and Ragupathy, 2010). This research represents the first time where the use of DNA barcoding in ethnobotany has been used to explore medicinal plants used by a diverse migrant community living in Western urban cities. A mixed methods approach combining social science research methods (i.e. questionnaires and interviews) and DNA barcoding techniques were used to fulfil the aims and objectives of this study.

5.1.1 Objective 1: Explore the knowledge and use of traditional herbal remedies amongst South Asian diasporic communities, in the UK; describe where the knowledge originates from.

While conducting the background research for this investigation, it became apparent that there was very little information on the use of traditional HMs by SA communities in the UK. Recent ethnopharmacological research has either looked at the general UK populations' perspectives of HMs (Ipsos MORI, 2008; Mintel, 2009), or focused on a specific gender, religious or ethnic group (Sandhu and Heinrich, 2005; Jennings, 2014). Hence, evidence of the current use of HMs amongst the wider SA population in the UK has been limited. Bhopal (1986a) conducted his research on the use of HMs by Asian communities over thirty years ago; the results of this study provide an important update on the use of HMs by SAs in the UK. The South Asian Traditional Medicines (SATMED) questionnaire was designed and administered by the primary researcher (Chapter 2), which enabled the collection of some valuable quantitative and qualitative primary data for this study.

The migration of SAs introduced different religions, traditions, cultures, cuisines, and traditional herbal remedies to the UK (Sandhu and Heinrich, 2005); yet little was known about the current use of HMs by SAs in the UK, until this research was conducted. Figure 5-2 illustrates the migration of SA participants to the UK, which may have been direct from South Asia (i.e. India, Pakistan, or Bangladesh) or via other parts of the world (e.g. Africa, Europe, The Middle East, Malaysia, Sri Lanka, etc.); it also summarises what participants brought to the UK with them (e.g. their knowledge and traditions of HMs) and the challenges participants faced in the UK (e.g. lack of knowledge of Western medicines, difficulty sourcing ingredients for HMs, and cultural/ language barriers).

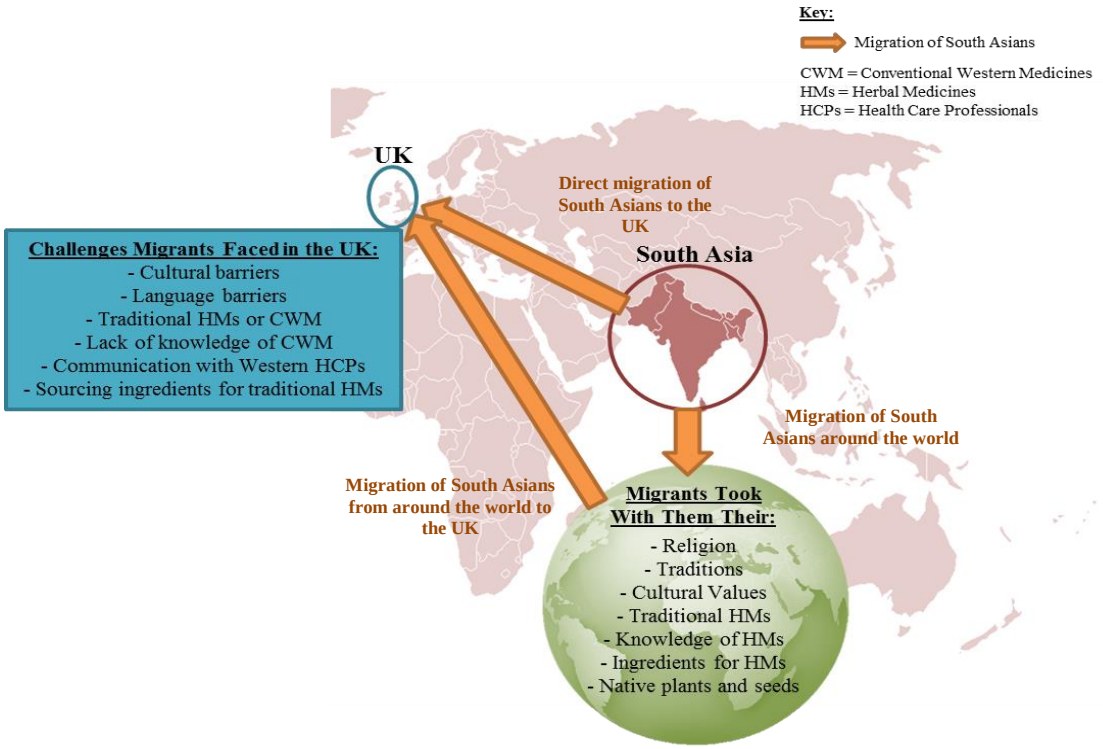


Figure 5-2 The Migration of South Asians to the UK - What Migrants Took with Them and Challenges They faced in The UK

The diagram represents the migration pattern of South Asians to the UK which may have been direct from South Asia or from other parts of the world where South Asians moved to. It also summarises what migrants took from their native country and the challenges they faced in the UK.

The knowledge of HMs has been passed on orally from generation to generation (Chhetri, 1994; Hatfield, 2007) and there is a risk of losing this knowledge if it is not documented (Thring and Weitz, 2006; Bhattia et al., 2014); this research has enabled some of this valuable information to be documented, as the vast range of ingredients, remedies, and products participants used for traditional HMs has been summarised (Appendix 5). Participants from the SATMED questionnaires (80%, n=101) and Tulsi Project interviews (62%, n=8) revealed that family was the primary source for transmitting the knowledge of traditional HMs; although, religion, the internet, television, and social media were emerging as alternative ways of learning about HMs. Hegg (2013) also identified the way people are learning about traditional HMs is changing, due to advances in technology and the popularity of social media.

The SATMED questionnaire revealed that there was a significant difference in knowledge of HMs between the genders (i.e. females knew more about HMs than male participants). Ernst (2000) claims that women generally use HMs more than men which may explain this difference in knowledge. Furthermore, gender differences in knowledge of HMs were also found by the Ipsos MORI (2008) and Mintel (2009) reports who claimed females had more knowledge of HMs than males.

A significant difference in knowledge of HMs was also identified amongst participants from the different age groups in this study (i.e. older participants had more knowledge than younger participants). Although younger participants may not have had as much knowledge of HMs in comparison to older participants, this may be due to a lack of experience which comes with age. Bhopal (1986a) predicted that the use of traditional medicines by young SAs would decline in the future as people became more familiar with Western medicines. The results of the SATMED survey identified that traditional HMs still play an important role in the lives of SAs of all ages in the UK. The results revealed that SAs participants of all ages use HMs to supplement and maintain their health, treat minor ailments, and manage side-effects caused by conventional Western medicines (CWM). It can be concluded from this research that traditional HMs still play an important role amongst SA communities in the UK as they are regarded as safe and effective and part of peoples' traditional heritage.

The Retail Think Tank (2013) identified that Western supermarkets, health stores, and online retailers are now stocking ethnic ingredients to cater for migrant communities. This was echoed by participants in this investigation who said ingredients for HMs which in the past were personally brought from abroad were now becoming commercially accessible in the UK. Participants identified some HMs, ingredients, dried plant material, and seeds (e.g. tulsi) which were still personally imported to the UK from abroad, mainly from India, Pakistan, and parts of Africa (e.g. Kenya and Uganda). This added to the interest of authentication of plant species in HMs which was explored further in Chapter 4 (Objective 3). As tulsi was one of the most popular products participants claimed to grow, import, and use for religious and medicinal purposes it was selected as the plant to be used for molecular analysis.

The use of HMs alongside CWM was also explored in the SATMED questionnaire, where SA participants (who can also be considered as patients) were asked about whether or not they shared the information about their use of HMs with their HCPs; 69% (n=73) of participants did not tell their doctor, while even more (82%, n=87) did not tell their pharmacist. Various reasons for not sharing information about the use of HMs with HCPs were noted including: the fear of being labelled as backwards or old fashioned, treated differently or not treated at all, HCPs not understanding problems patients presented with (i.e. due to language and cultural barriers), and not seeing their HCPs to be able to tell them. Similar results were disclosed by Eisenberg et al. (2001) where participants also reported feeling that HCPs did not understand them. Some participants in this study did not think it was important to share this information as HMs were regarded as being natural and therefore safe; this was also documented in the Ipsos MORI report (2008) where respondents did not think HCPs needed to know about their use of HMs. As patients' perspectives of traditional HMs were explored, this led to an interest in exploring the views and opinions of HCPs too.

5.1.2 Objective 2: Obtain insight into UK based healthcare professionals' knowledge and opinions of herbal medicines.

There are several examples of researchers who have looked into undergraduate medical or pharmacy students perspectives of HMs in the UK (Freymann et al., 2006; Pokladnikova and Lie, 2008; Smith, 2011). Numerous researchers have explored the views of HCPs in other countries such as the US, Europe, and Africa (Berman et al., 1998; Smith 2011; Awodele et al., 2012); yet, there is no current documented research which has investigated the opinions of HCPs in the UK. Research to explore the publics' perceptions of HMs in the UK conducted by Ipsos MORI (2008), on behalf of the Medicines and Healthcare products Regulatory Agency (MHRA), excluded HCPs from the research as they feared it would distort their results.

The lack of research into HCPs views of HMs may be restricted by ethical issues around recruiting HCPs to participate in research, as experienced during this investigation. Nevertheless, this study overcame the ethical concerns raised by the De Montfort University Ethics Committee and gained approval from the Leicester City Clinical Commissioning Group. This research used social media (i.e. Facebook and Twitter) to recruit participants and conduct the surveys online (using SurveyMonkey) to explore HCPs views of HMs.

Figure 5-1 summarises some of the key concerns HCPs raised about the use of HMs during this investigation, such as: the lack of clinical evidence to validate the safety and efficacy of HMs, the availability of poor quality HMs, and the lack of regulation of HMs which increases the risk of species substitution and adulteration. HCPs claimed that more research needs to be done on HMs and that they required more education on HMs before they could recommend them to their patients, or help patients make informed decision about HMs.

The SATMED questionnaire analysis uncovered several barriers which may have affected SA participants' willingness to share information about their use of HMs with their HCPs, such as language and cultural barriers; hence, many participants said they did not tell their HCPs about HMs they consumed. The Ipsos MORI report (2008) found that Asian and black and minority ethnic (BME) groups were less likely to tell their doctor about their use of HMs. The HCPs survey encompassed HCPs from all ethnic groups and their views on the general patient population not a single ethnic community. When HCPs were asked whether or not their patients informed them about their use of HMs 65% (n=64) claimed they did. From the finding of this research it is evident that cultural differences can affect the relationship between patients and their HCPs. In the *Tomorrow's Doctors* (GMC, 2009) standards for education guidelines published by the General Medical Council (GMC) for undergraduate medical students, it says students should have respect for cultural diversity

without prejudice. Hence, if HCPs were informed of alternative therapies different ethnic communities used it may help them better understand their patients and provide tailored advice and care.

The House of Lords report (2000) states that HCPs should be familiar with Complementary and Alternative Medicines (CAM), which is a broad term which includes hundreds of therapies including: acupuncture, reflexology, and HMs. Most regulatory bodies such as the GMC or General Pharmaceutical Council (GPhC) which govern HCPs and set standards for education of undergraduate courses also state CAM should be taught to students. However, Smith (2011) found that the level of CAM education varied significantly amongst medical schools in the UK. A large number of HCPs (65%, n=62) in this study claimed they did not receive any information about HMs during their professional training, this made them feel incompetent in giving advice to patients about the safe and effective use of HMs. The education of HCPs on CAM needs to be improved, as Smith (2011) suggests standardising the curriculum to ensure HCPs have some knowledge of HMs which patients may be using so they are competent to provide appropriate advice. Furthermore, guidance for patients to improve concordance with conventional medicine could be created to raise awareness of the implications of combining traditional and conventional medicines.

There was not enough up to date information on UK based HCPs views of HMs prior to this investigation being conducted. The results of this research can be used to contribute to this discipline where there is a need to improve HCPs knowledge and understanding of HMs used by the culturally diverse population in the UK. As the use of HMs continues to grow in the UK (Mintel, 2009) it is imperative for HCPs to understand the risks and benefits of consuming HMs alone or in conjunction with CWM as they could have a serious impact on patients' health.

5.1.3 Objective 3: Use DNA barcoding techniques for the identification of herbal medicines and medicinal plants used in South Asian communities.

From the results of the SATMED questionnaire tulsi (*O. tenuiflorum* L.) was identified as the medicinal plant of interest for DNA analysis. The HCPs survey results strengthened the purpose of conducting this authentication research, as HCPs highlighted the issue of species substitution and the need for more high quality research on HMs. This phase of the research was called the Tulsi Project and involved the multi-disciplinary collaboration of social science and molecular technology to attain the aims and objectives of the investigation. Interviews were conducted to explore the cultural and commercial value of the plant and tulsi samples were collected for molecular analysis; in total 111 tulsi samples including seeds, fresh and dried plant material, and commercial products (e.g. capsules, DNA extracts, oils, soaps, teas, and tablets) were accumulated during the research period.

The interviews revealed that the tulsi plant holds a prestigious position with Hindu families and temples in the UK, due to its religious affiliation with the Hindu goddess, Virinda Tulsi. In addition, the numerous health benefits mentioned by participants suggested the medicinal values were commonly utilised by participants for diabetes, asthma, respiratory infections, coughs and cold, and various skin conditions; these health benefits have been explored by many researchers (Miller and Miller, 2003; Prakash and Gupta, 2005; Winston and Maimes, 2007; Singh et al., 2012). The interviews enabled the collection of samples from participants personal tulsi plants (referred to as the community samples). Participants identified their tulsi plants as being either the Raam or Shyam varieties and disclosed where the seeds or plants were originally obtained from. Some participants claimed the seeds for their tulsi plants kept in the UK were either from India or Africa, and were commonly shared amongst family and friends. The DNA analysis exposed some interesting findings about the species of the tulsi samples collected from participants in the UK.

A combination of barcode regions should be used for the authentication of species (Li et al., 2011). The *matK* and *rbcL* regions have been referred to as the "*core barcodes*" for plants (Kress et al., 2005; Chase et al., 2005; Hollingsworth, 2011); however, they did not produce good quality sequencing data for many of the *Ocimum* samples in this investigation. The nuclear ITS and plastid *trnH-psbA* regions produced fast, efficient, and unambiguous results for several of the *Ocimum* species (e.g. *O. gratissimum* and *O. tenuiflorum*); thus, these regions were used to carry out the molecular analysis. Commercial products proved the most difficult to extract DNA from; hence, 29 samples were excluded from the analysis. In total

the species for 82% (n=67) of the samples analysed were successfully identified using ITS and *trnH-psbA* barcodes.

In comparison to other methods of medicinal plant authentication (e.g. morphology or chemical analysis) DNA barcoding is a relatively new technique which can be used to discriminate between species, and identify contaminants and adulterants (Kress et al., 2005; De Mattia et al., 2011). It was anticipated that the results of this research may provide a novel contribution to this discipline; this was successfully achieved in several ways. Firstly, there was a lack of reliable reference DNA sequences for the *Ocimum* genus in the current DNA databases (i.e. GenBank and BOLD); thus, authenticated *Ocimum* samples were obtained and reference DNA sequences were created which will be added to the databases for other researchers to use. Secondly, species substitution amongst the community Raam tulsi samples collected from participants in the UK was uncovered, as Raam and Shyam Tulsi are considered to be varieties of *O. tenuiflorum*; however, in this investigation Raam tulsi samples were identified as *O. gratissimum* instead. From the analysis of this research it was apparent that Raam tulsi samples collected in the UK had been substituted by alternative species. A small number of tulsi samples collected had been identified as being *O. basilicum* and several others as an African species (*O. gratissimum*) which may be linked to the migration routes of SAs to the UK (Chapter 4, Figure 4-9).

The results demonstrate that DNA based techniques are a valuable tool for the authentication of medicinal plant species. It can be argued that there is still more work to be done (i.e. to create more reference DNA sequences for other medicinal plant species); but, this rapidly evolving authentication technique has proved to be very powerful for the identification of species, contaminants, and adulterants in this research.

5.2 Limitations of the Research

5.2.1 Research Participants

5.2.1.1 Sample Size

With just over 3 million SAs living in the UK (Office for National Statistics, 2012) it would have been difficult to survey a significant number of participants in the time frame of this PhD as a single researcher. In addition, there is no validated or accessible sampling frame available for a sample to be drawn from. For these reasons a specific number of participants was not pre-determined and an opportunity sampling technique was employed to conduct the research. In the original PhD proposal for the SATMED questionnaire it was designed to be a self-completion questionnaire; however, after the pilot studies this was changed to be administered by the researcher. As this method of distribution was more time consuming it was not possible to complete as many questionnaires as anticipated (i.e. approximately three to four hundred).

The HCPs survey was completed online and circulated by participants which meant the researcher could not regulate the distribution or completion of the questionnaires. If surveys were conducted face to face it could have reduced the number of incomplete questionnaires (n=19) (Moore, 2000); however, this would have limited the range of HCPs recruited. As it was not possible to track who had received the link, attempted or completed the survey it was difficult to determine the potential response rate while the survey was available online. Despite the researcher forwarding the link to a large number of personal and professional contacts via several different channels (i.e. personal email, Facebook and Twitter accounts, Lloyds Pharmacy and Jhoots Pharmacy internal networks, and supervisory teams' contacts) the number of respondents could have been larger as only 112 participants attempted the survey.

For the Tulsi Project 13 interviews were conducted; these interviews provided an in depth insight into the topic and enabled very detailed responses to be collected from participants. Baker and Edwards (2012) claim the number of participants acceptable for a qualitative study could range from one to one hundred depending on the purpose of the research. After analysing the results of the 13 participants it was evident that the responses were very similar. Theoretical saturation had been reached as no new significant data were identified (Seale, 1999); therefore data collection was stopped. Despite the sample size being small it was sufficient to meet the objectives of the study (i.e. to determine the cultural values associated with the tulsi plant).

Generally in all three phases of the research (i.e. SATMED questionnaire, HCPs online survey, and Tulsi project interviews) more participants could have been recruited; but, the research needed to be conducted in a timely manner and as the aims and objectives of this research were successfully achieved the sample size was deemed sufficient.

5.2.1.2 Control Group of Participants for The SATMED Questionnaire

The aim of the SATMED questionnaire was to focus on SA participants, a population for which little ethnopharmacological data in the UK exists; other ethnic groups were not included in this investigation. However, if a control group of participants (i.e. not from SA ethnic backgrounds) were also included in this research comparisons between the use of HMs amongst different ethnic groups could have been made. As there was no intervention or experimental variables being explored in this study it was not necessary to have a control group (Punch, 2014).

Although the SATMED questionnaire only included SA participants the HCPs survey was open to people from all ethnic backgrounds. The HCPs survey analysis did reveal some interesting cultural differences in the use of HMs between participants from SA and other ethnic groups (Chapter 3).

5.2.2 DNA Analysis of Tulsi Samples

Before carrying out the DNA analysis it became apparent that there was a lack of reliable *Ocimum* reference DNA sequences for the study. Due to this, reference DNA sequences had to be created by first collecting reliable authenticated *Ocimum* specimens (e.g. seed, plant, and DNA samples) and then creating the DNA sequences. This took a significant amount of research time as samples for a range of different *Ocimum* species had to be obtained, seed samples needed to be germinated and grown, and then the DNA extraction and sequencing had to be done by the primary researcher.

One of the anticipated objectives of the Tulsi Project was to create a fast and reliable polymerase chain reaction (PCR) test to determine the type of *Ocimum* species (i.e. *O. basilicum*, *O. tenuiflorum*, or *O. gratissimum*) of samples; inspired by work conducted by Howard et al. (2009) for the identification of *Hypericum* species. If a PCR test to identify the *Ocimum* species was created it would allow for more samples to be analysed. However, due to time constraints and the time taken to collate and create the reference DNA sequences this was not possible. In retrospect this leads to further work which could be conducted as a result of this research.

5.3 Implications of the Research

Smith (2011) looked at the level of education of CAM amongst medical schools in the UK and found that despite it being a requirement of the GMC, the regulatory body which sets standards for education, the level of CAM education was highly variable (i.e. from a single lecture to student centred learning). A large number of HCPs in this study (74%, n=71) felt they did not have enough training on HMs. HCPs revealed that they felt incompetent to give patients advice on HMs as they lacked knowledge and said more training was required. If educational institutes could be made aware of the issues identified by participants in this research, changes to the curriculum could be implemented to improve the level of CAM education. This would mean future HCPs are better equipped with the knowledge of HMs to help make better clinical decisions and inform patients about the safety and efficacy of HMs. HCPs are often required to undertake Continuing Professional Development (CPD) as part of their professional practice. After giving a presentation to the Biomolecular Technology Group at De Montfort University the potential to create a CPD programme for HCPs was discussed with the supervisory team and Gary Trappit (Business Development Manager at DMU); this will be considered at a later date.

Participants in the Tulsi Project revealed that the tulsi plant was kept for various reasons including religious and medicinal purposes. Both Raam and Shyam varieties of tulsi were kept by people in the UK and collected for molecular analysis. During the DNA analysis of tulsi samples collected from participants, it was uncovered that Raam tulsi plants kept in the UK were not *O. tenuiflorum* but were *O. gratissimum* instead. This leads to several questions which the researcher would have liked to explore further including: What are the implications of having the wrong species (i.e. medicinally and religious/culturally), and whether or not the species of tulsi matters to people who keep it for religious or cultural purposes. Questions for a focus group to explore these concepts further were designed (Appendix 16). The university ethical approval delayed the process and due to time constraints this was not completed. This research has provided the background and resources to continue the work in the future.

One of the desired outcomes of this research was to design a fast and efficient PCR test to distinguish between *Ocimum* species (Howard, 2010). As the starting material were collected during this research and the reference DNA sequences were created, this work was successfully completed at De Montfort University by a visiting researcher Seethapathy GS from the Ashoka Trust for Research in Ecology and the Environment, India (July 2015). This will allow for a larger study to authenticate the species of tulsi samples.

5.4 Conclusion

This research has presented the results of the unique integration of ethnopharmacological research with DNA analysis something which (to my knowledge) has not been previously been done before. The results of this thesis could have important implications for the range of disciplines studied. The SA population in the UK is rising (Office for National Statistics, 2012), now this research has documented evidence which reveals: the knowledge of HMs SA people have, types of HMs they use, and where ingredients are sourced from. The results demonstrate that traditional HMs are still commonly used by diasporic SA communities in the UK. This study enabled both patient and practitioners' view of HMs to be explored. HCPs raised concerns about the safety and efficacy of unregulated HMs which lacked reliable clinical evidence, and felt they needed more training on the risks and benefits of HMs. Ultimately, if HCPs were better educated about HMs they would be able to deal with patients' queries about HMs competently, ensuring patients received the best pharmacological care. The unregulated import of HMs, products, ingredients or seeds, to the UK carries risk of species substitution and adulteration which was demonstrated in the Tulsi Project. The molecular work conducted highlighted the usefulness of DNA analysis in authenticating plant species in HMs, and proved to be valuable technique for fast and efficient identification of the *Ocimum* species.

The multi-disciplinary approach used to conduct this research has enabled the perspectives of SA communities and Western HCPs of HMs in the UK to be explored, as well as the use of DNA barcoding in ethnobotany; providing a unique contribution to ethnopharmacology.

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Appendices

Appendix 1 Research Proposal for Traditional Herbal Remedies PhD Project

**Please refer to the external memory device for the complete SATMED Ethical Proposal*

Appendix 1 contains:

1. Standard ethics application form for the SATMED project
2. Ethics proposal- SATMED version 1
3. Ethical Approval Application, Amendments
4. Ethics proposal- SATMED version 2
5. Ethical Approval Amendments – for version 3
6. Ethics proposal- SATMED version 3

Appendix 2 Ethical Approval Communications

Appendix 2A Ethics Proposal Amendment Form for HCPs Questionnaire

De Montfort University

Faculty of Health & Life Sciences

Faculty Research Ethics Committee

AMENDMENT/EXTENTION APPLICATION FORM

PLEASE READ SUBMISSION GUIDELINES BEFORE COMPLETING THIS FORM

Further information and application forms are available at http://www.dmu.ac.uk/research/ethics-and-governance/faculty-specific-procedures/health-and-life-sciences-ethics-procedures.aspx.

For further information or advice please contact the Research and Commercial Office, Faculty of Health and Life Sciences, 1.25 Edith Murphy House, Phone: 0116 2506122 / 0116 2577891 or email: hlsfro@dmu.ac.uk

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Amendments may include changes to the terms of the FREC application, the protocol or any other supporting documentation for the trial. Applicants should consider;

a) Any change in the research proposal
b) The safety or physical or mental integrity of the subjects of the trial
c) The scientific value of the trial
d) The conduct or management of the trial
e) The quality or safety of any investigational medicinal product used in the trial

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1. Applicant name:

Sukvinder Kaur Bhamra

2.Postal & email address:

93 Brooklands Road
Birmingham
B28 8LB
suky_bhamra@hotmail.com

3. Supervisor(s) or co-applicants:

Prof Adrian Slater, Prof Mark Johnson, Dr. Caroline Howard

4. Programme (if applicable):

n/a

5. Title of Research Project:

Investigating the use and identity of traditional herbal remedies amongst South Asian communities using biomolecular techniques.

6. Original Approval date for the project:

Reference number;

111

3

7. Start date for the project

01/10/2012

8.Expected end date for the project:

(FREC/RCO must be advised upon completion)

October 2015

9. Brief description of proposed amendment

One of the objectives set in the initial proposal (version 3) was 'to gain an insight into healthcare professionals knowledge and understanding of traditional herbal medicines used in South Asian communities in the UK'. A questionnaire and Participant Information Leaflet (PIL) has now been created to fulfil this objective.

The questionnaire has been designed to gain an insight into healthcare professionals' personal and professional opinions of traditional herbal medicines. The term healthcare professionals (HCPs) refers to those who have regular clinical contact with patients. The main focus for this study will be on General Practitioners (GPs) and pharmacists as they are at the frontline of dealing with minor health problems which may be treated with herbal medicines. In addition, for the purpose of this exploratory research other HCPs who may also have had opportunities to discuss herbal medicines with their patients will also be given the chance to participate (i.e. including nurses, dentists and opticians).

The questionnaire will be administered as a postal questionnaire to GPs and Pharmacists across Birmingham and Leicester, whose addresses are readily available in the public domain (i.e. directories/ the internet). Hence, the style of the PIL is similar to a cover letter. Survey monkey (an online survey software) will also be used to publish the same questionnaire online in anticipation of reaching more people and getting a larger response rate. An opportunity sampling technique will be employed as healthcare professionals known to the research team will be asked to participate and forward the link for the online survey to other healthcare professionals to complete.

10. Rationale for the amendment

Page 6 of the ethics proposal highlighted that separate ethical approval will be sought at a later stage depending on the progress of the study. After assessing the progress of the study, it is now at a stage where this objective can be explored.

11. Please list each potential ethical issue relating to your study and state how these will be addressed: include potential risks to participants and research staff

The Participant Information Leaflet (PIL) has been carefully worded to prevent harm being caused to participants. A PIL produced by the GPhC was used as a template to tailor this to healthcare professionals.

Healthcare professionals who use traditional herbal medicines may feel hesitant to share this information but anonymity and confidentiality will be assured.

All postal questionnaires will be returned to De Montfort University and not the researchers personal address to ensure personal details of the researcher are kept safe.

Appendix 2B HCPs Questionnaire Ethics Review Reply

Re: Ethical Approval Application – Investigating the use and identity of traditional herbal remedies amongst South Asian communities using biomolecular techniques. (Ref: 1113)

Reviewers comments:	Applicants response:
*I was unable to find any information in the application paperwork about who is included in the definition '**healthcare professionals**' and how they will be **recruited**. The proposal doesn't seem to have been extended from the original to include details about this section of the study - **sample size, inclusion criteria, recruitment methods etc**. so it is difficult to give an ethical opinion on the work to be completed. This links directly to the issue **of NHS R&D approval**. If the research is proposing to recruit NHS healthcare professionals, we might expect to gain this. I am assuming this is not the intention, but clarification needs to be sought on whether it is needed - dependent on the sample and the means of recruitment. We need an update on this specific phase of the study and clarification of whether NHS R&D approval is required.*	**Clarification of Healthcare Professionals:** The term healthcare professionals (HCPs) refers to those who have regular clinical contact with patients. The main focus for this study will be on General Practitioners (GPs) and pharmacists as they are at the frontline of dealing with minor health problems which may be treated with herbal medicines. In addition, for the purpose of this exploratory research other HCPs who may also have had opportunities to discuss herbal medicines with their patients will also be given the chance to participate (i.e. including nurses, dentists and opticians). Not only are we are looking for healthcare professionals who may have experience of discussing the use of herbal medicines with patients but we also want to know their personal views and opinions of herbal medicines. **Recruitment methods:** The postal questionnaire will only be sent to GP surgeries and pharmacies whose addresses are readily available in the public domain (i.e. directories/ the internet). Once the target locations have been agreed (with supervisors) we will collate a list of address to post the questionnaires to. If ethical approval is granted I will seek advice from my supervisor (Prof. Mark Johnson) and DMU staff who have experience with this form of questionnaire distribution (e.g. Peter Rivers, Sandra Hall etc.). An online version of the questionnaire which will be published via Survey Monkey can be completed by any healthcare professional if they are interested in participating. An opportunity sampling technique will be employed as healthcare professionals known to the research team will be asked to participate and forward the link for the online survey to other healthcare professionals to complete. **Sample size:** There are no defined parameters for the sample size as this exploratory research is an open survey which will be running in the background while the main themes of the research are explored. The survey will be available online for a year, from the date of publishing the questionnaire, as this is the duration of the license we will purchase for the online survey software. **Inclusion criteria:** Participants must be over 18 years old so participants can consent for themselves. Participants must be a healthcare professional, either practicing or retired. The healthcare practitioner must have practiced in the UK at some point in their career (no specific time period). The rationale for posting questionnaires to GP surgeries and pharmacists is because these healthcare professionals are the first port of call in a community setting (i.e. primary care) who will deal with minor ailments which may often be treated with herbal medicines. Hence we are most interested in the views and opinions of GPs and

	pharmacists. However, as this is an open survey with the intention to explore healthcare professional's views and opinions of herbal medicines anyone who is a healthcare professional and wishes to participate can do so. This is so we can get a variety of healthcare professional's views and opinions of herbal medicines. **NHS R&D approval:** Advice from several colleagues from DMU and external NHS ethics advisors has been useful in clarifying whether or not NHS R&D approval would be required. Including: Maggie Barrett - Senior RM&G Facilitator (Leicestershire, Northamptonshire & Rutland Comprehensive Local Research Network). Debbie Wall - Research & Development Manager, NHS Leicester City (awaiting a response). As we will not be using the NHS to recruit participants or conduct/ host the research; we have no sponsors and will not require any patient details we would not require NHS ethical approval. I am currently working on completing the IRAS forms to get assurance from Debbie Wall to clarify this. *'Research involving staff as participants REC review is not normally required for research involving NHS or social care staff recruited as research participants by virtue of their professional role.' - See more at: http://www.hra.nhs.uk/resources/before-you-apply/research-requiring-nhs-rd-review-but-not-ethical-review/#sthash.QIjBvS5h.dpuf* ***Exceptions to the need for REC review*** *Subject to any overriding legal requirements, REC review is not required for the following types of research:* *-Research limited to the involvement of NHS or social care staff recruited as research participants by virtue of their professional role.* *- Research involving use of or access to a care organisation's premises or facilities, but not otherwise involving patients or service users. - See more at: http://www.hra.nhs.uk/resources/research-legislation-and-governance/governance-arrangements-for-research-ethics-committees/#sthash.LkrVSzts.dpuf*
In relation to ethical issues raised, the researcher has not indicated ***what they would do if a participant were to reveal dangerous or poor practice*** *in this questionnaire.*	The questionnaire will be anonymous as we are not asking for any personal/ identifiable details (for example registration/ license details are not required). If dangerous practices were identified there would be no way of identifying the participant so we wouldn't be able to address them even if they were identified. The nature of this questionnaire has been designed with the intent of exploring the healthcare professionals' views and experience with herbal medicines, not their professional practice.

Appendix 2C NHS R&D Approval Letter

NHS
Leicester City
Clinical Commissioning Group

NHS
West Leicestershire
Clinical Commissioning Group

NHS
East Leicestershire and Rutland
Clinical Commissioning Group

City Headquarters
St John's House
30 East Street
Leicester
LE1 6NB

Tel: 0116 295 1478
www.leicestercityccg.nhs.uk

06.05.2014

Study Ref: LCCR 200214

End Date: 31.03.2015

Project Status: Approved

Dear Miss Sukvinder Bhamra

Re: Investigating the use of traditional herbal remedies

I am pleased to confirm that Leicester City Clinical Commissioning Group which provides the Primary Care R&D Service across Leicester, Leicestershire and Rutland, has received a review of this research study via the Coordinated System for gaining NHS Permission (CSP) from the Clinical Research Network: East Midlands. This review confirms that the appropriate study-wide and local research governance checks have been undertaken and that this study complies with the requirements of the Research Governance Framework and national legislation. In conjunction with the local discussions the Clinical Research Network: East Midlands have had with you regarding participating as a site for this study, I am now happy to formally provide assurance for this study to proceed within Leicester City CCG, East Leicestershire & Rutland CCG and West Leicestershire CCG. Your research has been entered onto our primary care research database.

This assurance is provided with the following conditions: that no additional costs are incurred at the site (s) as a result of this study being undertaken and unmet service support costs will be provided by the Clinical Research Network: East Midlands.

Please reply to this letter confirming the expected start date and duration of the study. As part of the Research Governance Framework it is important that the Leicester City CCG R&D office is notified as to the outcome of your research. As such the Clinical Research

Network: East Midlands will request feedback once the research has finished along with details of dissemination of your findings. We may also request brief updates of your progress from time to time, dependent on duration of the study. Similarly, if at any time details relating to the research project or research team change, the Leicester City CCG R&D office must be informed.

The documents reviewed for assurance are as follows:

Document Title Version Date REC

Approval DMU Faculty Research Ethics Committee approval letter N/A 25.06.13 N/A

Amendment 1 1.0 October 2013 28.11.2013

Ethics Proposal – SATMED 3.0 N/A 25.06.13

Participant Information Leaflet – Healthcare Professionals 3.0 October 2013 28.11.2013

Questionnaire – Healthcare Professionals 3.0 October 2013 28.11.2013

If you have any further questions regarding this or other primary care research you may wish to undertake in the Leicester, Leicestershire and Rutland area, please feel free to contact me again, or the Clinical Research Network: East Midlands Primary Care research office. I wish you every success with the research.

Yours sincerely
Deb Wall
R&D Manager
Leicester City CCG
Tel: 0116 295 1520
Email: debbie.wall@leicestercityccg.nhs.uk

Appendix 2D Ethics Proposal Amendment Form For The Tulsi Project

DE MONTFORT UNIVERSITY

APPLICATION FORM FOR RESEARCH ACTIVITY REQUIRING HUMAN RESEARCH

ETHICAL CONSIDERATION OR APPROVAL

Applicant name:	**Postal address and contact details:**
Sukvinder Kaur Bhamra	93 Brooklands Road, Hall Green, Birmingham, B28 8LB Tel: 07828246920 Email: p07246383@myemail.dmu.ac.uk

Supervisor(s) (if applicable):	**Programme (if applicable):**
n/a	
Prof. Adrian Slater, Prof. Mark Johnson, Dr. Caroline Howard	South Asian traditional Indian herbal medicine (SATMED)

Title of Research Project:

A study to explore the evolving role of tulsi, *Ocimum tenuiflorum*, within a multicultural population, followed by the genetic authentication of samples collected.

Start date for the project:	**Expected end date for the project:** **(FREC/RCO must be advised upon completion)**
01/10/2012	October 2016

Brief description of proposed activity and its objectives:

The tulsi project has emerged as a satellite project, which has developed during the initial stages of my PhD. Extensive background research done suggests that this plant (*Ocimum tenuiflorum*) has multiple medicinal benefits, huge cultural significance and would be a great starting point to develop my biomolecular analytical skills.

From this project we anticipate collecting a variety of tulsi samples from participants, whether it be tulsi seeds to grow in the growth room, leaves (fresh/dry) or any other tulsi product which we can extract DNA from. These will be added to the current traditional herbal medicines inventory we have in the university. At the same time as collecting samples of tulsi we will ask participants a few questions, a semi-structured interview, about their tulsi. Questions will aim to gain an insight into where their tulsi is from, why it is kept, what it is used for and any other significant facts about the plant.

Please see the attached proposal for further information*.

**Please refer to the external memory device for the complete Tulsi Project Ethical Proposal*

Ethical issues identified:	How these will be addressed:
Confidentiality & anonymity.	Responses will be coded with a reference number to protect participants' identity. No data will be identifiable once coded and analysed.
Risk of causing distress to participants.	Wording of questions will be considered carefully to prevent any adverse effects on participants.
Informed consent.	A participant information leaflet will be provided to all participants prior to the interview; this will enlighten participants as to what the research is about and enable them to give informed consent. Participants will have the freedom to withdraw at any point during the interview.
Unauthorised access to data collected.	Researcher will keep all data collected in a lockable cabinet, with restricted access. All data on computers and memory sticks will be password protected.

To which ethical codes of conduct have you referred? These are specific to each Faculty/School, if you have a query please ask your supervisor or Faculty REC for advice.

The British Sociological Association has been consulted for ethical guidance in this instance. http://www.britsoc.co.uk/media/27107/StatementofEthicalPractice.pdf

**Please refer to the external memory device for the complete Tulsi Project Ethical Proposal*

Appendix 3 SATMED Questionnaire

Questionnaire on the Use & Knowledge of Traditional Herbal Medicine

Q1a. Do you use any herbs, plants or spices to make homemade herbal remedies for your health?

Yes ☐
No ☐

If your answer is No please go to question 6

Q1b. If **YES**, what do you use and why?

.....
.....
.....

Q2. Where do you get the ingredients for your herbal remedies from?

Tick all that apply.

Family/ friends ☐
Grow your own ☐
Ayurvedic clinic ☐
Local supermarket ☐
Abroad ☐
Online ☐
Other ☐
Please state:.....

Q3. What form of herbal remedies do you use?

Tick all that apply.

Fresh herbs ☐
Dry powders ☐
Seeds ☐
Tablets ☐
Liquid formulations ☐
Creams / Pastes ☐
Other
Please state:……..

Q4. After using herbal remedies do you notice any unwanted effects?

Yes ☐
No ☐

If YES, please state:
.....
.....
.....

Q5. Where did you learn about herbal remedies?

Family/ Friends ☐
GP/ Pharmacist ☐
Educational institute e.g. school ☐
Other ☐
Please state:.....

Q6. Is herbal medicine a part of your traditional family & cultural background?

Yes ☐
No ☐

Q7a. How often do you use herbal medicinal products?

Daily ☐
Weekly ☐
Monthly ☐
When required ☐
Never* ☐

If your answer is Never, please go to question 8.

Q7b. What **herbal products** do you use for medicinal purposes?
Please state what they are used for:

……
……
……

Q8a. List as many **herbal remedies**, **products or ingredients** you know of:

.....
.....
.....
……
……
……
……

Q8b. What **plants** do you know that have medicinal benefits?
Please list the plants and what they are used for.

……
……
……
……
……

Q9. Do you know any herbal remedies or products which can be used for the following conditions?

Condition:	**Herbal remedy:**
Arthritis	
Cough	
Diabetes	
Headache	
Indigestion	
Itchy skin	
Wounds	

Prescription Medicines:

Q 10a. Do you take any regular prescribed medicines? Yes ☐ No ☐

If NO please go to question 13

Q 10b. If **YES**, and you are happy to do so please state what medicines you take:
Otherwise please go to question 11

.....
.....

Q11a. Do you tell your **Doctor** what herbal remedies you use? Yes ☐ No ☐

Q11b. Do you tell your **pharmacist** what herbal remedies you use? Yes ☐ No ☐

Q 12a. Do you notice any unwanted effects from your regular medicines? Yes ☐ No ☐

Q 12b. If **YES** what do you normally do to relieve the symptoms?

Use a herbal medicine ☐
Get a product over the counter from your pharmacy ☐
See the doctor for a medicine ☐
Nothing ☐

If you have any concerns regarding any of your medicines please see your doctor or local pharmacist.

Minor illness: what would you do?

Q13. Do you use herbal remedies for minor health problems before seeking medical help?

Yes ☐
No ☐

For question 14 and 15 please rank in the order you are most likely to do first.
1 being the most likely action you would take followed by 2, 3 and 4 for the least likely.

Q14. In which order would you consider the following treatments for a minor illness.

E.g. A cold/ sore throat/ migraine

Please rank 1-4

a. Would not consider taking any treatment. ☐
b. Self-medication using herbal products. ☐
c. Self-medication using over the counter products from the pharmacy. ☐
d. Consult your GP for advice and medication. ☐

Q15. Who are you most likely to seek advice from for minor health problems?

Please rank 1-4

a. Family/ Friends ☐
b. Pharmacist ☐
c. Doctor ☐
d. Herbal practitioner ☐

Q16. Would you **prefer** to use traditional herbal remedies over conventional western medicines for minor health problems?

Yes ☐
No ☐

Q17. Which do you think are more **effective** for treating **minor** health conditions?

Traditional herbal remedies ☐
Prescription medication ☐

Q18. Which do you think are more **effective** for **treating serious, long term** health conditions?

Traditional herbal remedies ☐
Prescription medication ☐

About you:

* Gender: Male ☐ Female ☐

* What is your age range? 20 or under ☐ 21-30 ☐ 31-40 ☐ 41-50 ☐ 51-60 ☐ 61+ ☐

* What is your ethnic origin? Asian – Indian ☐ Asian – Pakistani ☐ Asian- Bangladeshi ☐

Other please state:.....

* Place of birth: United Kingdom ☐ India ☐ Africa ☐ Pakistan ☐

Other please state:…….

* Parents place of birth: Mother……..

Father……

* What languages are spoken at home?

1st ……...
Other ……..

* What is your religion? Hindu ☐ Sikh ☐ Muslim ☐ Christian ☐ No religion ☐

Other, please state:.....

* What is your highest level of qualification?.....

* What is your job role?……

* What is your postcode *(first 4 digits)* ……

Thank you for completing this questionnaire

Appendix 4 SATMED Participant Information Leaflet

Traditional Herbal Medicine - Participant Information Leaflet

My name is Sukvinder Kaur Bhamra. I am doing a PhD at De Montfort University in traditional herbal medicine. I have a keen interest in traditional herbal medicine as it encompasses the use of herbs and spices for medicinal purposes.

My aim is to explore people's knowledge and use of traditional herbal remedies and to discover how this knowledge has been transmitted through generations. After carrying out this research I aim to gain an insight into the variety of herbal products available and used within the community.

In order to participate in this research you must be over eighteen years old, you will need to complete this questionnaire, which will take no more than fifteen minutes. By completing the questionnaire you are consenting for the data to be analysed and published as part of my thesis, academic journals and conferences.

If you wish to withdraw from participation, you may discard your questionnaire before handing it back to me. Once you hand in a completed questionnaire there will be no way of identifying your responses, so there will be no way of withdrawing your answers from the study. As no personal details are required the process ensures anonymity.

All completed questionnaires will remain confidential as no unauthorised person will have access to the data.

If you would like to be part of future research done at the university on traditional herbal medicine then please ask to leave your contact details and we may be in touch.

I hope you will enjoy this and thank you for participating in my research.

.....

For further information please do not hesitate to contact:

Prof Adrian Slater: ads@dmu.ac.uk

Suky- Sukvinder Bhamra: suky_bhamra@hotmail.com

Appendix 5 Summary of Herbal Medicines Recalled by SATMED Participants

Traditional name:	Commonly known as:	Scientific name:	Therapeutic uses:	Methods of consumption:	Other comments:
Adarak / Sond	Ginger	*Zingiber officinale*	Arthritis Colds Digestion Pain Inflammation	-Mix with honey, hot water, and lemon -Chew it whole -Used in cooking -Infuse in water/tea	*Garam*
Aritha	Soapnut tree	*Sapindus saponaria*	Cough, helps to reduce excessive mucus and phlegm	-Warm in some milk	-
Alsi /ulsi	Linseed/ flaxseed	*Linum usitatissimum*	Arthritis Pain Strength/ energy Cholesterol Diabetes Constipation	-Make into a mixtures called *pajiri* mixed with fruits and nuts -Roast on a hot pan and grind into a powder and take a spoon with water/ chew	*Garam*
Ajwain	Carum seeds/ Bishop's weed	*Trachyspermum ammi*	Digestive aid Indigestion Headache Constipation	-Seeds to be chewed -Infused in tea -Soaked in water -Used in cooking	*Garam* Chew seeds with some salt
Amla/ olay	Indian gooseberry	*Emblica officinalic*	Healthy hair Strength/ stamina Digestion	-Fruit preserved in syrup can be eaten -Herbal formulations available e.g. oil and tablets	Found in *Triphala*
Anjirhi	Figs	*Ficus carica*	Constipation Diabetes	-Infused in milk -Drink the juice	Available in pharmacies too
Badam	Almonds	*Prunus dulcis*	Energy Mental stamina/ memory Skin and hair vitality	-Eat a few daily/ soak in water before eating -Used in cooking -Oil massaged on skin or in hair	Very expensive in India, we take them from UK
Baheray	Belleric	*Terminalia bellirica*	Digestion	-Juice -Fruit preserved in sugar syrup -Herbal formulation	Fruit is used. Found in *Triphala*

Bajri (Bajra)	Millet flour	*Eleusine coracana*	Diabetes	-Used to make chapattis instead of wheat flour	Healthier chapattis
Banaksha	English violet	*Viola odorata*	Coughs and colds	-Syrup -Tablets -Infused in tea/ hot water	Imported from India and Pakistan
Besan	Gram flour	*Cicer arietinum*	Skin cleanser Healthy alternative to wheat flour	-Used in cooking -Used to make face masks, make a paste with yoghurt and lemon	Made from ground chick peas
Bindi	Okra	*Abelmoschus esculentus*	Diabetes	-Infused in water overnight -Used in cooking	-
Chana dhal	Chick peas	*Cicer arietinum*	Source of protein General health	-Used in cooking	-
Chasku	Four leaved cassia	*Cassia absus*	Blood tonic/ purifier	-Seeds soaked in water overnight	Very *garam*
Dalchini	Cinnamon	*Cinnamomum verum*	Arthritis Cough Colds Digestion Cholesterol	-Infused in water/ tea -Used in cooking -Mixed with other herbs and eaten -Mix with honey	Very *garam* Can induce menstrual flow and nose bleeds
Elachi (Bhadi)	Black cardamom (Big)	*Elettaria cadamomum*	Nausea Indigestion Digestion Reduces phlegm and mucus	-Chew whole -Infused in tea or water -Used in cooking	Black cardamoms are *garam*, green ones are not *garam.*
Elachi (shoti)	Green Cardamom (small)	*Amomum subulatum*			
Ghanda / pyaaz	Onion	*Olea europaea*	Sickness Arthritis General health	-Juice an onion and drink if feeling sick -Rub onto affected joints for pain relief -Used in cooking	Core of the onion is harmful, can cause cancer so do not use.
Gulab jal	Rose water	*Roas sp*	Eye health & vision Skin cleanser Reduces spots	-Instil drops into eyes -Apply to skin	-
Gulkand	Sweet preserved rose petals	*Rosa sp*	Eye health Constipation Digestion	-Eaten as required -Used in cooking	-

Haldi	Turmeric	*Curcuma longa*	General health Arthritis Pain relief Inflammation Immune boosting effects Wound healing Skin conditions Cancer Sore throats/ cough	-Mixed with milk, or hot water. -Make a paste using oil or water -Mix with honey and chew for a cough, or apply as a paste for skin problems	*Garam* Can add *ghee*, olive oil or black pepper to liquid formulations to improve absorption.
Hardar	Black Myrobalan or Indian Gall Nut	*Terminalia chebula*	Constipation Digestion Liver protection Diarrhoea	-Fruit preserved in sugar syrup -Powder taken with milk/ water -Herbal formulations	Fruit used. Found in *Triphala* and *Chyawanprash*
Hing	Asafoetida	*Ferula assa-foetida*	Digestion Reduces gas	-Used in cooking	*Garam*
Jaifal	Nutmeg	*Myristica fragrans*	Digestion	-Used in cooking	-
Jamun	Indian blackberry	*Syzygium cumini*	Diabetes Blood circulation and purification	-Eat the fresh or dried fruit	Juice and tablets available
Jeera	Cumin seeds	*Cuminum cyminum*	Arthritis	-Used in cooking -Dry roast in hot pan and chew/ mix with other ingredients	-
Kachur	White turmeric	*Curcuma zedoria*	Blood purification Tonic	-Used in cooking -Drink powder dissolved in water	-
Kadu	Pumpkin	*Cucurbita maxima*	Diabetes Eye health Cancer	-Used in cooking	-
Kaju	Cashew	*Anacardium occidentale*	Strength Essential fats	-Eat a few daily -Used in cooking	*Garam*
Kala Jeera	Black cumin	*Bunium persicum*	Digestive aid	-Used in cooking	-
Kali mirch	Black pepper	*Piper nigrum*	Cough Digestion Vision/ eye problems	-Mix with honey, hot water, and lemon -Chew it whole -Used in cooking	*Garam*
Karoth	Walnuts	*Juglans regia*	Arthritis Cholesterol Diabetes Essential fatty oil	-Eat a few daily -Grind and mix with other ingredients	-

Kalonji	Onion / black seeds	*Nigella sativa*	Arthritis Pain Diabetes	-Seeds incorporated in cooking -Oil rubbed onto affected joints	God given remedy by the Prophet Mohamme d known to cure all disease
Kapuraveli leaves	-	*Plectranthus amboinicus* Synonym: *Ocimum vaalae*	Colds Congestion	-Soaked in water with black pepper & sugar candy for sore throat	-
Karela	Bitter gourd	*Momordica charantia*	Diabetes Blood purifier Vitamins	-Used in cooking -Fresh karela juiced -Juice & tablets are available	Very powerful anti-diabetic. Too much can be harmful
Kaskas	Poppy seeds	*Papaver somniferum*	Arthritis/ Pain Digestion Diarrhoea	-Chew seeds -Infuse in tea/ water -Used in cooking	-
Kesar	Saffron	*Crocus sativus*	Enriches blood Digestion	-Used in cooking -Infuse in milk	-
Kickar	Cape gum	*Acacia karoo*	Colds & flu Oral hygiene	-Bark used to clean teeth -Infuse in water/ milk/ tea	-
Kori/ pahri saunf	Dill seeds	*Anethum graveolens*	Digestion	-Used in cooking -Take a pinch with water or chew it	-
Kwar	Aloe vera	*Aloe vera*	Arthritis Burns Diabetes Eczema Itchy skin Skin conditions	-Apply sap into skin -Juice to drink -Used in cooking -Herbal formulations available (tablets, creams)	*Garam*
Lassan	Garlic	*Allium sativum,*	Antibacterial Earache Arthritis Digestion Skin conditions Thins the blood Improves circulation	-Infuse in oil and put into the ear for ear infections or apply to painful joints -Used in cooking -Clove rubbed directly on to the skin	*Garam* Anti-bacterial properties
Lauki	Bottle gourd	*Lagenaria siceraria*	Diabetes Digestion Weight loss	-Juiced -Used in cooking	Good to boost energy if unwell

Limbro	Curry leaf	*Murraya koenigii*	Digestion Diabetes	-Used in cooking	-
Long	Clove	*Syzygium aromaticum*	Pain Headache Toothache	-Infused in tea -Place a clove next to a painful tooth	-
Malathi	Liquorice	*Glycyrrhiza glabra*	Cough Digestion	-Root boiled in tea -Herbal tablets -Sweets available	Bad for high blood pressure. *Garam*
Methi	Fenugreek	*Trigonella foenum-graecum*	Arthritis Diabetes Digestion	-Leaves used in cooking -Seeds infused in water	*Garam*
Mirch	Chilli pepper	*Capsicum annuum*	Pain Digestion	-Make a paste with salt and turmeric and apply to joints. -Used in cooking	-
Mooli	Radish	*Raphanus sativus*	Diabetes Cancer Blood pressure	-Eaten raw -Used in cooking	-
Moringa	Drumsticks	*Moringa Oleifera*	Arthritis Diabetes Vitamins & minerals	-Soaked in water over night -Used in cooking	Very nutritious
Naaryal	Coconut	*Cocos nucifera*	General health Hair health and growth Skin conditioning	-Used in cooking -Oil applied to hair or skin Available in many forms including oil, juice, milk, fresh and dry fruit – versatile use.	*Garam* Too much can cause nose bleeds
Neem/ Nim	Neem	*Azadirachta indica*	Blood purifier Diabetes Skin conditions	-Infused in water/ tea -Leaves rubbed onto the skin or infused in bath water -Tablets -Juice	*Garam* and can cause internal dryness must take with oil or ghee
Nimbu	Lemon	*Citrus limon*	Cough Sore throat Skin lighter Spots & pimples	-Mix juice with honey and hot water (can add cinnamon, ginger or black pepper) -Rub lemon slice over skin to lighten or remove scars -Drink the juice or infuse in water	People with arthritis shouldn't use lemons.

Olive	Olive	*Olea europaea*	Arthritis Skin conditions e.g. eczema Ear infections and wax	-Apply oil to skin -Used in cooking -Warm oil and instil drops into ears (can add garlic for infections)	Used as a base for many formulations
Paan leaf	Betel leaf	*Piper betle*	Digestion	-Leaf is used to wrap ingredients which make Paan	-
Palak	Spinach	*Spinacia oleracea*	Source of iron Good for the blood and internal strength	-Used in cooking	-
Pipal	Sacred fig	*Ficus religiosa*	Diarrhoea Digestion Liver health	-Leaves used to treat wounds -Infused in tea/ water	-
Pista	Pistachio	*Pistaci vera*	Cholesterol General health	-Eat daily -Used in cooking	*Garam*
Puthna	Mint	*Mentha arvensis*	Digestion Indigestion	-Leaves infused in water or tea -Used in cooking	-
Ramphal	Custard apple	*Annona reticulata*	Diarrhoea Cancer Immune boosting properties	-Consume the fruit	Full of vitamins and minerals
Saron	Mustard	*Brassica juncea*	Pain Dry skin	-Apply oil to affected areas -Seeds used in cooking	Oil used as a base for numerous remedies
Saunf (sweet saunf)	Fennel	*Foeniculum vulgare*	Bloating Constipation Cough Digestion	-Chew seeds -Infuse in tea	'Cold' so can cause bhye
Soggi	Raisins	*Vitus vinifera*	Blood purifier Cough	-Infused in water -Chewed -Used in cooking	*Garam*
Suphari	Betel nut	*Areca catechu*	Digestion Stimulant	-Nut is crushed and chewed -Incorporated in paan -Used in cooking	Linked to causing cancer
Tej patha	Bay leaf	*Laurus nobilis*	Diabetes Digestion	-Used in cooking	-
Thil	Sesame seeds	*Sesamum indicum*	Warms blood Improves circulation Reduce urinary frequency	-Used in cooking -Mix seeds with jaggery -Oil to be applied topically	*Garam*

Thora/ kori buti	Bugleweed	*Ajuga bracteosa*	Gout Menstrual problems	-Infused in water	-
Thunia or dhania	Coriander	*Coriandrum sativum*	Digestion Diabetes	-Leaves used in cooking -Seeds infused in water	-
Tulsi	Holy Basil	*Ocimum tenuiflorum*	Asthma Coughs and colds Diabetes Antibacterial	-Chew leaves -Infuse in tea/ water -Rub on to skin -Capsules, creams, oil& juice available	Can cure any disease as it represents a goddess. Avoid in pregnant women.

Other products

Traditional name:	**Commonly known as:**	**Scientific name:**	**Therapeutic uses:**	**Methods of consumption:**	**Other comments:**
Amrat dhara	Polyherbal formulation. Contains ajwain, fennel, camphor and other herbals.	Not applicable	Colds Headache	-Rub onto forehead/ temples	Imported from Pakistan
Churan Brand: *Hajmola*	Polyherbal formulation. Contains black pepper, black salt, ginger, cumin, lemon and other herbals.	Not applicable	Constipation Digestive aid	-Powder -Tablets	Imported from Asia but now available to buy in the UK
Chyawanprash	Polyherbal formulation of between 25-80 ingredients including amla, ghee, asparagus, cardamom, cinnamon and many other herbals.	More than 32 herbals combined	Strength & nourishment Source of vitamins & minerals	-Eat a spoonful of the solid mixture daily	Have it with milk to make it more palatable
Dushanda	Polyherbal formulation	Not applicable	Colds and flu Cough	-Dried material infused in hot water -Tea and Juice are available	Imported from India and Pakistan
Dhud	Milk	Not applicable	Source of calcium Healthy bones	-Drink daily -Used in tea and cooking	A base for many other ingredients e.g. turmeric or cinnamon
Fatkari	Alum	Not applicable	Skin conditions e.g. spots/acne	-Formulations available for skin conditions	-

Ghee	Clarified butter	Not applicable	Lubricates joints Pain Wounds Constipation	-Used in cooking -Paste with turmeric for wounds and pain -In hot milk for constipation	-
Gor	Jaggery	Not applicable	Substitute sugar in diabetes	-Used in cooking -Used as a base for many other herbal formulations	Makes remedies more palatable
Jal (Ganga jal)	Holy water	Not applicable	Heals all ailments	-Drink the water -Apply to affected areas	Can cure all illnesses if god is willing
Kala loon	Black salt	Not applicable	Digestion Constipation	-Sprinkle black salt over food/ fruit -Mix with ajwain	Very punjent smell
Kapoor	Camphor	Not applicable	Pain relief Migraine	-Eaten raw, or with some milk	A waxy substance that is used during Hindu prayers
Katha	Paste applied to paan leaves	Not applicable	Digestion	-Used to flavour and line a paan leaf	-
Masala (Garam masala)	Polyherbal formulation	Not applicable	Arthritis Digestion Pain (menstrual pain)	-Make tea with it in -Used in cooking	*Garam*
Munaka	Golden raisin	-	Coughs Sore throat	-Chew the dried fruit	-
Mishri	Crystallised sugar	Not applicable	Sore throat Cough	-Chew with tulsi or black pepper	-
Semian	*Vermicelli*	Not applicable	Colds & flu Energy and nutritional	-Cook with milk and ghee	-
Shilajeet	-	Not applicable	Blood circulation & purification Libido Mental & physical stamina	-Mix with warm milk and drink -Crush dried resin into a powder and mix take half a teaspoon with water	It is as expensive as gold and difficult to obtain, as it comes from the mountains in India.

Appendix 6 Healthcare Professionals Questionnaire

Healthcare Professionals' Views of Traditional Herbal Medicines

Your personal use of Herbal Medicines:

1. Is traditional herbal medicine part of your family/ cultural background?

- (•) Yes
- ( ) No

2. Do you use any plants, herbs or spices for your own health?

- ( ) Yes
- ( ) No

*If Yes, what do you use and why?

_____
_____
_____

3. Would you prefer to use traditional herbal remedies over conventional Western medicine for minor health problems?
E.g. cold/constipation/mild skin irritation?

- ( ) Yes
- ( ) No

4. Can you name any herbal remedies which can be used for the following conditions? -If so, please state what could be used.

Arthritis_____
Cough_____
Diabetes_____
Indigestion_____

Patients' use of Herbal Medicines:

5. Do you currently have contact with patients?

- (•) Yes
- ( ) No

6. Do your patients tell you about any traditional herbal medicines they use?

- ( ) Yes
- ( ) No
- ( ) Not applicable

7. How often do you come across patients who take traditional herbal medicines alongside their prescription medicines?

- Daily
- Weekly
- Monthly
- Annually
- Never

8. Have you encountered any adverse effects caused by the consumption of traditional herbal medicines?

- Yes
- No

*If Yes, please give a brief description:

_____
_____
_____

9. Are you aware of any interactions between traditional herbal medicines and conventional Western medicines?

- Yes
- No

*If Yes, please give a brief description:

_____
_____
_____

Professional opinion of Herbal Medicines:

10. Do you think the use of plants, herbs and spices is effective to prevent or treat various health conditions?

- Yes
- No

11. Do you think the use of plants, herbs and spices is safe?

- Yes
- No

12. Would you ever recommend herbal medicines to your patients?

- Yes
- No

*If Yes, what would you recommend and why?

_____
_____
_____

13. Did you receive any information about herbal medicines during your professional training?

- Yes
- No

14. Do you think enough training is provided to you about herbal medicines?

- Yes
- No

15. Any other comments:

_____
_____
_____

About you:

16. Gender:

- Male
- Female

17. Age range:

- 20 or under
- 21-30
- 31-40
- 41-50
- 51-60
- 61+

18. Ethnic origin:

- White: British
- White: Irish
- Black/ Black British: African
- Black/ Black British: Caribbean
- Asian/ Asian British: Indian
- Asian/ Asian British: Pakistani
- Asian/ Asian British: Bangladeshi
- Other

Other (please specify) _____

19. Place of birth:

- United Kingdom
- India
- Pakistan
- Bangladesh
- Africa

Other (please specify) _____

20. Religion:

- No religion
- Christian
- Hindu
- Sikh
- Muslim

Other (please specify)_____

21. Highest level of qualification:

_____

22. Job role:

_____

23. Do you or have you ever worked in the UK?

- Yes
- No

If Not, please specify: _____

Appendix 7 Healthcare Professionals Information Leaflet

My name is Sukvinder Bhamra; I am doing my PhD in Ethnopharmacy at De Montfort University. I will be exploring the use of traditional herbal remedies by South Asian diasporic communities in the UK.

Nature has provided a pharmacy, which people have recognised and taken full advantage of. The knowledge of which plants are medicinally beneficial comes from the use of plants as food, trial and error, and now more commonly from scientific research. Many Western medicines available have been developed from natural sources; approximately 25% of medicines prescribed worldwide are derived from plants. The use of plants, herbs and spices to prevent and treat diseases goes back before written records were created and this continues to thrive today.

As healthcare professionals we may be faced with many challenges; including the concurrent use of conventional western medicine and traditional herbal medicine. Is it safe or effective to consume herbs and spices for medicinal purposes?

Have your say

Here is the chance to share your views anonymously. Here is the opportunity to express your personal and professional opinions on traditional herbal medicines.

Taking part

The questionnaire is designed to explore UK based healthcare professionals' views and opinions on traditional herbal medicines. In order to participate all you have to do is answer a few questions, which will take no more than ten minutes. By completing the questionnaire you are consenting for the data to be analysed and published as part of my thesis, in academic journals and at conferences.

Confidentiality

All completed questionnaires will remain confidential as no unauthorised person will have access to the data. As no personal details are required the process ensures anonymity and there will be no way of identifying your responses. This study has been approved by the De Montfort University Ethics Committee and NHS Leicester City, Clinical Commissioning Group.

Sukvinder Bhamra MPharm
PhD researcher in Ethnopharmacy

Appendix 8 Tulsi Project Interview Questions

Tulsi Project Interview Questions

Identification of the sample:	
Sample ID number:	
Name of sample donor:	
Contact details of donor:	
Details of sample give: **No of leaves/ seeds etc**	

1.	Where did you get your Tulsi from?	
2a.	Do you have any Tulsi seeds?	
2b.	Do you grow your own Tulsi?	
3.	What type of Tulsi is this? (Raam/ Krishna/ Vana/ other?)	
4.	Can you tell me all the different names Tulsi has:	
5.	Do you keep Tulsi in your home? If so why? (i.e. for religious reasons/ just for its medicinal purposes/ no particular reason just have it)	
6.	Where did you learn about Tulsi? (e.g. family –mother/ grandparents, religious teachings, other…)	
7.	Does it have a religious purpose in your life? If so what is it?	
8.	Are there any special requirements for storing/ growing/ keeping Tulsi? If so what are they? (e.g. don't touch at night, keep in one place…)	
9.	What do you use the Tulsi for? (Any medicinal purposes/ cooking?)	
10.	What are the medicinal benefits of using Tulsi? If it is used for medicinal purposes what are the formulations used/ how is it prepared/ other ingredients used to make the remedy? (please list as many as you can think of)	
11.	Is there any other information which you think is important/ interesting to know about Tulsi? (e.g. Tulsi Vivah/ any rituals- pray to it twice a day etc)	

About you:

*What is your gender?

Male ☐
Female ☐

*What is your age range?

Under 20 ☐
21-30 ☐
31-40 ☐
41-50 ☐
51-60 ☐
61 + ☐

*What is your ethnic origin?

Asian – Indian ☐
Asian- Bangladeshi ☐
Asian – Pakistani ☐
Black- African ☐
Black- Caribbean ☐
Chinese ☐
White ☐

Other please state:.....

*What is your religion?

Christian ☐
Hindu ☐
Muslim ☐
Sikh ☐
Other ☐

Please state:…….

*Place of birth:

United Kingdom ☐
India ☐
Africa ☐
Pakistan ☐
Other ☐

Please state:……..

*Parents place of birth:

Mother: ……..

Father: ……

Appendix 9 Tulsi Project Participant Information Leaflet

Tulsi Project Participant Information Leaflet

My name is Sukvinder Kaur Bhamra. I am doing a PhD at De Montfort University in traditional Indian herbal medicine. I have a keen interest in tulsi, Holy basil, *Ocimum tenuiflorum*.

Tulsi *'the queen of herbs'* is one of India's most sacred herbs known for its divine healing properties. The tulsi plant holds a prestigious position in every Hindu household, as it represents the goddess Virinda Tulsi; a woman of immense purity and devotion to God.

My aim is to explore the origins of tulsi, why people keep it in their homes, what they use it for and to collect a variety of samples for DNA analysis, to verify the genetics of the plant. I would also like to collect tulsi seeds to add to our current tulsi nursery.

In order to participate in this research you must be over eighteen years old, you will need to answer some questions, which will take no more than fifteen minutes. By answering the questions you are consenting for the data to be analysed and published as part of my thesis. If you wish to withdraw from participation, you may choose to stop the interview at any point.

If you have any tulsi products and are happy to give me a sample I only need a small amount. If you are happy to be contacted at a later date for further information please leave your name and contact details. All samples collected will be given a reference number so anonymity will be ensured throughout the process. All information disclosed will remain confidential as no unauthorised person will have access to the data.I hope you will enjoy this and thank you for participating in my research.

.....

For further information please contact:
Prof Adrian Slater (Supervisor): ads@dmu.ac.uk
Sukvinder Bhamra: p07246383@myemail.dmu.ac.uk

Appendix 10 Tulsi Project Promotion Poster

DE MONTFORT UNIVERSITY LEICESTER

THE TULSI HUNT IS ON!

DO YOU HAVE ANY TULSI PRODUCTS AT HOME OR KNOW SOMEONE WHO DOES?

THEN WE WANT TO KNOW!

WE ARE INTERESTED IN THE MEDICINAL BENEFITS OF TULSI, THE RELIGIOUS SIGNIFICANCE THE PLANT HOLDS AND HOW THIS KNOWLEDGE HAS BEEN TRANSMITTED THROUGH GENERATIONS.
IF YOU HAVE ANY TULSI PRODUCTS AND WOULD BE HAPPY TO SHARE THEM WITH US, ALONG WITH ANSWERING A FEW QUESTIONS THEN **WE NEED YOU.**

WE WANT TULSI:

- PLANTS
- LEAVES (FRESH/ DRY)
- SEEDS
- ANY TULSI PRODUCTS:
  - TEA BAGS
  - BEADS
  - COSMETICS

EVENTS WILL BE HELD NEAR YOU SOON.
LOOK OUT FOR YOUR CHANCE TO SHARE YOUR TULSI.
EVERY PARTICIPANT WILL BE ENTERED INTO PRIZE DRAW OF **WINNING** A TULSI HAMPER FILLED WITH TULSI PRODUCTS AND A PLANT GROWN AT THE UNIVERSITY.

GET IN TOUCH FOR FURTHER INFORMATION OR LOG ONTO
WWW.DMUTULSIPROJECT.WEBS.COM
CONTACT SUKY ON:
EMAIL: P07246383@MYEMAIL.DMU.AC.UK
LEAVE YOUR NAME AND CONTACT DETAILS AND WE WILL BE IN TOUCH.

Appendix 11 Tulsi Project Leaflet

Appendix 12 Summary of the *Ocimum* Samples and their Identity

*Species identification was based on the reference DNA sequences created using the reference Ocimum specimens obtained combined with the reference sequences from GenBank. Identification was based on a 100% match across the sequence length after the ends were trimmed to start and end at the same position (*some species identified according to the closest match on GenBank).*

Please refer to the external memory device for the sample sequences

Key:

- = No result
n/a = Not applicable as not analysed
Grey text = Sample identification failed
* = Species identified according to the closest match on GenBank

Category:	Sample ID:	Extraction Number:	Sample source:	Type of tulsi specified:	Type of Sample:	Species identification – ITS:	Species identification - *trnH-psbA:*
Community Tulsi samples	**B01**	**432**	K.K	*TBC*	Fresh leaves	*O. gratissimum*	*O. gratissimum*
	B02a	**433**	H.P	*Raam*	Fresh leaves	*O. gratissimum*	*O. gratissimum*
	B02b	**425**	H.P	*Shyam*	Fresh leaves	*O. gratissimum*	*O. gratissimum*
	B03	**435**	A.S	*TBC*	Seeds	*Veronica species**	*Veronica species**
	B04	**436**	R.M	*Raam*	Fresh leaves	*O. basilicum**	*O. basilicum**
	B05	**437**	L.M	*Raam*	Fresh leaves	*O. gratissimum*	*O. gratissimum*
	B06	**438**	L.P	*Shyam*	Fresh leaves	*O. tenuiflorum*	*O. tenuiflorum*
	B06g	**431**	L.P	*Shyam*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	B07	**422**	SSHC	*Raam*	Fresh leaves	*O. gratissimum*	*O. gratissimum*
	B07g	**439**	SSHC	*Raam*	Seeds	*O. gratissimum*	*O. gratissimum*
	B09	**697**	K.P India	*TBC*	Dried leaves	-	*n/a*
	L10	**459**	M.M	*TBC*	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
	L11	**460**	M.A	*TBC*	Dried leaves	*O. basilicum**	*O. basilicum**
	B12	-	B.C	*TBC*	Seeds	*n/a*	*n/a*
	A14	**490**	D.N	*TBC*	Seeds	*O. gratissimum*	*O. gratissimum*
	B15a	**491**	H.A	*Raam*	Dried leaves	*O. gratissimum*	*O. gratissimum*
	B15b	**492**	H.A	*Shyam*	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
	Li79	**840**	K.R	*TBC*	Dried leaves	-	*O. tenuiflorum*
	Li80	**841**	M.J	*Basil*	Dried leaves	-	*O. basilicum**
	U100	**892**	A.S	*TBC*	Dried leaves	-	*O. tenuiflorum*
	B101	**893**	Apna ghar	*TBC*	Dried leaves	–	–
	B102 a	**947**	R.P	*Shyam*	Dried leaves	-	*O. tenuiflorum*
	B102 b	**950**	R.P	*Shyam*	Dried leaves	-	*O. tenuiflorum*
	B102 c	**948**	R.P	*Raam*	Dried leaves	-	*O. tenuiflorum*
	B102 d	**949**	R.P	*TBC*	Dried leaves	-	*O. tenuiflorum*
	B105	-	R.R	*TBC*	Seeds	*n/a*	*n/a*
Commercial Tulsi	**B00**	424	Tesco basil	*O. basilicum*	Fresh leaves	*O. basilicum**	*O. basilicum**
	C08	423	Pukka three tulsi tea bag	*Raam, Shyam & lemon tulsi*	Tea bag	-	*O. tenuiflorum*

	C13	461	Shyam tulsi seeds ebay	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	C16	493	Akamba garden centre	*O. basilicum*	Fresh leaves	*O. basilicum**	*O. basilicum**
	C20	574	Pukka	*O. tenuiflorum*	Capsule	-	-
	C21	575	Pukka	*O. tenuiflorum*	Oil	-	*n/a*
	C22	576	Pukka	*TBC*	Tulsi beads	-	*n/a*
	C23	615	Pukka	*Raam*	Dried leaves	-	-
	C24	616	Pukka	*Shyam*	Dried leaves	-	-
	C25	617	Pukka	*Lemon tulsi*	Dried leaves	-	-
	C40	-	Herbal Veda	*O. tenuiflorum*	Tulsi juice	-	*n/a*
	I44	786	Fort Kochi	*TBC*	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
	I45	787	Phalada Agro	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
	I46	789	Phalada Agro	*O. tenuiflorum*	Dried leaves	*O. tenuiflorum*	*O. tenuiflorum*
	I47	790	Phalada Agro	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
	I48	791	Phalada Agro	*O. tenuiflorum*	Dried leaves	-	*O. tenuiflorum*
	I49	792	Phalada Agro	*TBC*	Dried leaves	-	-
	C50	0110	Organic India	*O. tenuiflorum*	Capsules	-	*n/a*
	C51	0111	Organic India	*O. gratissimum*	Capsules	-	*n/a*
	C52	0112	Organic India	*O. gratissimum*	Capsules	-	*n/a*
	C53	0113	Organic India	*O. tenuiflorum*	Capsules	-	*n/a*
	C54	0114	Organic India	*O. tenuiflorum & gratissimum*	Capsules	-	*n/a*
	C55	0115	Organic India	*O. tenuiflorum & gratissimum*	Capsules	-	*n/a*
	C56	0120	VASU respiratory care	*O. tenuiflorum*	Capsules	-	*n/a*
	C57	0121	Bipha pharmacy	*O. tenuiflorum*	Tablets	-	*n/a*
	C58	0122	Sudarsanam	*O. tenuiflorum*	Tablets	-	*n/a*
	C59	0123	SAINA	*O. tenuiflorum*	Tablets	-	*n/a*
	C60	0124	MUCEX	*O. tenuiflorum*	Tablets	-	*n/a*
	C61	0125	Madhu mardan	*O. tenuiflorum*	Powder	-	*n/a*
	C62	0126	Sitopaladi Churna powder	*O. tenuiflorum*	Powder	-	*n/a*
	C63	0128	VASU	*O. tenuiflorum*	Syrup	-	*n/a*
	C64	0129	Cruz cough syrup	*O. tenuiflorum*	Syrup	-	*n/a*
	C65	0130	Krishna Tulsi cough syrup	*O. tenuiflorum*	Syrup	-	*n/a*

	C66	0131	Amrutha Kasturo cough syrup	*O. tenuiflorum*	Syrup	-	*n/a*
	C67	0132	Bipha Tulsi essential oil	*O. tenuiflorum*	Oil	-	*n/a*
	C68	0133	KPN Ayurvedic Hair oil	*O. tenuiflorum*	Oil	-	*n/a*
	C69	0134	Krishna Tulsi Hair Tonic	*O. tenuiflorum*	Oil	-	*n/a*
	C70	0315	Dabur	*O. tenuiflorum*	Toothpaste	-	*n/a*
	C71	-	Bipha Neem and Tulsi soap	*TBC*	Soap bar	-	*n/a*
	C72	-	Bipha Tulsi Soap	*TBC*	Soap bar	-	*n/a*
	C73	834	Pukka	*TBC (Raam)*	Dried leaves	-	*O. tenuiflorum*
	C74	835	Pukka	*TBC (Raam)*	Dried leaves	-	*O. tenuiflorum*
	C75	836	Pukka	*TBC (Krishna)*	Dried leaves	-	-
	C76	837	Pukka	*TBC (Krishna)*	Dried leaves	-	*O. tenuiflorum*
	C77	838	Pukka	*TBC(lemon vana)*	Dried leaves	-	-
	C78	839	Pukka	*TBC (lemon vana)*	Dried leaves	-	*O. basilicum*
Miscellaneous Tulsi samples	**A17**	571	Egypt	*TBC*	Dried leaves	*O. basilicum**	*O. africanmum**
	A18	572	Egypt	*TBC*	Dried leaves	-	*O. basilicum**
	A19	573	Egypt	*TBC*	Dried leaves	-	*O. africanmum**
	I41	783	Taj Hotel India	*O. tenuiflorum*	Dried leaves	-	*O. basilicum**
	I42	784	Taj Hotel India	*O. tenuiflorum*	Dried leaves	-	*O. basilicum**
	I43	*785*	Taj Hotel India	*O. basilicum*	Dried leaves	-	*O. basilicum**
Reference *Ocimum* samples	**G26**	618	Peter Nick	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	G27	619	Peter Nick	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	G28	620	Peter Nick	*O. africanum*	Seeds	-	*O. africanum*
	G29	674	Peter Nick	*O. citridorum*	Seeds	-	*O. citridorum**
	G30	768	Peter Nick	*O. gratissimum*	Seeds	*O. gratissimum*	*O. gratissimum*
	G31	676	Peter Nick	*O.kilimandscharicum*	Seeds	-	*O.kilimandscharicum**
	G32	769	Peter Nick	*O. gratissimum*	Seeds	*O. gratissimum*	*O. gratissimum*
	G33	678	Peter	*O. tenuiflorum*	Seeds	-	-

			Nick				
	G34	745	Peter Nick	*O. basilicum*	Seeds	*O. basilicum**	*O. basilicum**
	G35	842	Peter Nick	*O. carnosum*	Seeds	-	-
	G36	746	Peter Nick	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	G37	770	Peter Nick	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	G38	771	Peter Nick	*O. tenuiflorum*	Seeds	*O. tenuiflorum*	*O. tenuiflorum*
	G39	844	Peter Nick	*O. tenuiflorum*	Seeds	-	*O. tenuiflorum*
	C81	1138	Kew	*O. gratissimum*	DNA extract	*O. gratissimum*	*O. gratissimum*
	C82	1583	Kew	*O. selloi*	DNA extract	*O. selloi**	-
	C83	9936	Kew	*O. basilicum*	DNA extract	-	-
	C84	9937	Kew	*O. tenuiflorum*	DNA extract	-	*O. tenuiflorum*
	C85	9939	Kew	*O. americanum*	DNA extract	-	*O. americanum**
	V86	865	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V87	866	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V88	867	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V89	868	CAMAG	*O. tenuiflorum*	Dried material	-	-
	V90	869	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V91	870	CAMAG	*O. americanum*	Dried material	-	-
	V92	871	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V93	872	CAMAG	*O. tenuiflorum*	Dried material	*O. tenuiflorum*	*O. tenuiflorum*
	V94	873	CAMAG	*O. tenuiflorum*	Dried material	*O. tenuiflorum*	*O. tenuiflorum*
	V95	874	CAMAG	*O. tenuiflorum*	Dried material	-	-
	V96	875	CAMAG	*O. tenuiflorum*	Dried material	-	*O. tenuiflorum*
	V97	876	CAMAG	*O. tenuiflorum*	Dried material	-	-
	V98	877	CAMAG	*O. tenuiflorum*	Dried material	*O. tenuiflorum*	*O. tenuiflorum*
	V99	878	CAMAG	*O. tenuiflorum*	Dried material	*O. tenuiflorum*	*O. tenuiflorum*

Appendix 13 DNA Sequences and Multiple Alignments

**Please refer to the external memory device for DNA sequences and multiple alignments created during this research.*

All figures included in the thesis can be viewed in pdf format.

Sequences, contigs and additional multiple alignments can be viewed using a trial version of CLC sequence viewer which can be downloaded free from:
http://www.clcbio.com/products/clc-sequence-viewer/

Appendix 14 *Ocimum* Sequence Accession Numbers from GenBank

ITS

Ocimum americanum GU810517.1; *Ocimum basilicum* DQ667240.1; *Ocimum basilicum* GU810522.1; *Ocimum basilicum* KF815492.1; *Ocimum bascilicum* x *Ocimum gratissimum* GU810521.1; *Ocimum gratissimum* FJ593402.1; *Ocimum gratissimum* GU810520.1; *Ocimum gratissimum* GU810518.1 *Ocimum gratissimum* GU810516.1; *Ocimum gratissimum* GU810515.1; *Ocimum selloi* JF301405.1; *Ocimum tenuiflorum* GU810519.1

trnH-psbA

Ocimum africanum JX294428.1; *Ocimum africanum* JX262182.1; *Ocimum africanum* KP218940.1; *Ocimum americanum* JX262179.1; *Ocimum basilicum* DQ667350.1; *Ocimum basilicum* KF855613.1; *Ocimum basilicum* JX262185.1; *Ocimum basilicum* JQ339256.1; *Ocimum campechianum* HG963580.1; *Ocimum filamentosum* JX294429.1; Ocimum gratissimum JX262181.1; *Ocimum gratissimum* FR726109.1; *Ocimum gratissimum* KF855614.1; *Ocimum kilimandscharicum* JX262180.1; *Ocimum tenuiflorum* cultivar Krishna tulsi JX262184.1; *Ocimum tenuiflorum* cultivar Ram tulsi : JX262183.1; *Ocimum tenuiflorum* FR726110.1;

Appendix 15 *Ocimum* Species Summary Table

Image:	Species:	General Description:	Description of Leaves:	Description of Flowers: (Inflorescence)	Description of Fruit:	Native to:	Any other information:	DNA Sequence:
	***Ocimum africanum***	-Annual herb -Erect much branched -Aromatic -0.3-1m tall -Stem and branches are quadrangular -Hairs on stem grow side-wards and downwards = spreading	-Simple decussate with petiolate. -lance to elliptical shape -Hairless -Glands on both upper and lower surface	-	-4 nutlets -Black -Nutlet wall produces a thick white cover in water	Tropical Africa and Asia	Often confused with *O. basilicum* Synonym*:* *O. americanum* *O. canum* *O. brachiatum*	G28_620 ITS ACTGCAAGCAGACCGCGAACACGTG TTTAACTCATGAATCCCGCCGCCGGC CCCGGTCGGCGCGCGGGCTAACGAA CCCCGGCGCGAACCGCGCCAAGGAA AACTCAAAACGTCGGCGGCGGCCCC CCCCACCCCGTCCCGCGGTCGTGCG GGGGGCGCGGCCTCCATCGACGAA TGTCAAAACGACTCTCGGCAACGGA TATCTCGGCTCTCGCATCGATGAAGA ACGTAGCGAATGCGATACTTGGTG TGAATTGCAGAATCCCGTGAACCAT CGAGTCTTTGAACGCAAGTTGCGCC CGAAGCCATCAGGCCGAGGGCACGT CTGCCTGGGCGTCACGCATCGCGTC GCCCCCCATCCCGCGAGAATGCGCT CGGCTTGGGGGGCGGATACTGGCC TCCCGTGCGCCCCGCCGGCGGGCCG GCCCAAATGCGATCCCCGCGCGAGC CGCGTCGGCAACAGTGGTGGTTGAA CTCATCAATTTCCCCGTCGCGGCCCG GGTCGTCCGCGCGGATTCCTTTAATAA AAAGAACCCAAGCTCCTCGACCGCG ACCCCAGGTCAGGCGGGATCACCCG TTGAGTTTAAC *trnH-psbA* TAATGCTCATAACTTCCCTCTAGACC TAGCTGCTATTGAAGCTCCAACAAAT GGCTAAAACTTGTTCTTGGTGTGTAG AAGTTTTTGAAATATAGATAAATATA AGGAGCAATAAACCCTTTCTTGTTCT ATCAAAAGAGGTTTATTGCTCCTTT CTTTTATTTTCAATTAGAAGTCCTTT GTATTTTCTAGTAGTATTGGACTTA CCTAGACTTTTCTTTCCATAAAAGCA AGAAGATAAAAAATGATTCAAATT CTATCTTTTGCTTTTGTTTTACAATTT TGAAAAAAAAAAATTCAAAAAGTG AATTCGTAAATAAATAGAAAATTTA CTTTTTTAGAATAGTGGGGCGGA
	***Ocimum basilicum***	-Annual herb -0.3-0.5m tall up to 1m -Almost hairless plant = glabrous If there are any hairs they only grow in one direction =side-wards	-Silky green opposite (paired) oval leaves -No hairs -Highly aromatic with glands on surface of the leaf. -Fleshy leaves -Convex shape -Very few hairs on leaf/ stem	-White or -Pink -Large flowers	Fruits have four small nutlets, which are mucilaginous when wet	Tropical origins	Closely related to and frequently confused with *O. africanum* and *O. americanum*, but they can be identified on the basis of indumentum (hair distribution) and flower size. Lemon-scented cultivars are usually the result of crosses between *O. basilicum* and *O. africanum*.	G34_745 ITS ACTCATGAATCCCGCCGGCCCCC GGTCGGCGCGGGGCTAACGAACCC CGGCGCGGAACGCGGCAAGGAAAAC TCAAAACGTCGGCGCCGGCCCCCCC CCCCCCTTCCCCGGGTCGGGGGGGG GGGGCGGGGCTCCATCGACGAATGT CAAAACAACTCTCGGCAACGGATAT CTCGGCTCTCGCATCTATGAAAAAA GTAGCGAAATGCGATACTTGGTGTG AATTGCAGAATCCCGTGAACCATCG AGTCTTTTAACGCAAGTTGCGCCCGA AACCATCAGGGCGAGGGGACGTCTG CCTGGGCGTCACGCATCGCGTCGCC CCCCCCTCCCCGGCGAATGCGCTCGG GTTGGGGGGGGGGATACTGGGCTCC CGTCGCCCCCGCCGGCGGCCGGCC CAAATGCGATCCCCCGGGGACCCGC GTCGCGACAAGTGGTGGTTGAACTC ATCAATTTCCCCGTCGCGCCCGGGT CGTCCGGCGGGATCCTTTTTTAAAAA AAACCCAACCTCCTCGACCCCCACC CCAGGTGGGGCGGCATCACCCGTTT ATTTTAATCATAT *trnH-psbA* TCCGCCCCACTATTCTAAAAAGTA AATTTCTATTTATTTAACGAATTCAC TTTTTGAATTTTTTTTTTTCAAAATT GTAAAACAAAAGCAAAAGATAGAAT TTCAATCATTTTTTATCTCTTGCTT TTATGGAAAGAAAAGTCTAGGTAAG TCCAATACTACTAGAAAAATACAAA AGGACTCTAATTGAAAATAAAAGA AAGGAGCAATAAACCCTCTTTTGAT AGAACAAGAAAGGGTTTATTGCTCC TTATATTTATCTATATTTCAAAAACT TCTACACACCAAGAACAAGTTTTAG CCATTTGTTGGAGCTTCAATAGCAGC TAG

Image:	Species:	General Description:	Description of Leaves:	Description of Flowers: (Inflorescence)	Description of Fruit:	Native to:	Any other information:	DNA Sequence:
	***Ocimum citridorium***	-20-40cm tall -Lemony -Spreading hairs	-Leaves similar to basil but flatter -Strong citrus fragrance like lemons -Pointer leaves -Thinner leaves AP	-White flowers	-	Northeast Africa and southern Asia	A hybrid from basil- *O. basilicum* and African basil- *O. americanum* Smells very lemony	G29_674 ITS CGTGTTTAACTCATGAATCCCGCCGCCGCCGGCCCGGTCGGCGCCGGGCTAACGAACCCCGGCGCGGAACGCGGCAAGGAAAACTCAAAACGTCGCGCCGCGGCCCCCCCCCCCCCCCCCCGGGGTGGGGGGGGGGCGGGCGTCCCTCGACAAATGTCAAAAAACTCTCTGCAACAGATATCTCGGCTCTCGCATCTATAAAAAAATAGAAAAAGAGATATTTGGTGTGAATTGTAGAATCCCCGAACCATCGAGTTTTTAACACAAGTTGTCCCCCAAAACATCATGGCGAGGGGGGGTCTGCCTGGGGGTCACACATCGCGTCGCCCCCCCTCCCCCCGGCAATGCGCTCTGGTTGGGGGGGGGGATATTGGGCTCCCGTGGGCCCCCCCGCGCGGGCGGCCCAAATGTGATCCCCCCGCCAACCCCCTCGCGACAAGTGGGGTTGAAAACATCAATTTTTCCCCCGCGCCCCCGGGGGTCCGCGCGGATCCTTTTTTAAAAAACAACCCTCTCCCACCGCCCCCCGGGGTGGGGGGGTATCCCCC *trnH-psbA* CATCCGCCCCACTATTCTAAAAAGTAAATTTTCTATTTATTTACGAATTCACTTTTTGAATTTTTTTTTTCAAAATTGTAAAACAAAGATAGAATTTCAATCATTTTTATCTTCTTGCTTTTATGGAAAGAAAAGTCTAGGTAAGTCCAATACTACTAGAAAATACAAAGGACTTCTAATTGAAAATAAAGAAAGGAGCAATAAACCCTCTTTTGATAGAACAAGAAAGGGTTTATTGCTCCTATATTTATCTATATTTCAAAAACTTCTACACACCAAGAACAAGTTTTAGCCATTTGTTGGAGCTTCAATAGCAGCTAGGT
	***Ocimum gratissimum***	-Herbaceous plant. -Perennial herb. -1-3m tall with an erect stem. -Highly branched which is smooth or Hairy. -Woody base	-Bright green leaves, -Large and flat egg shaped leaves -Larger leaves compared to O. tenuiflorum.	Greenish white petals	4 dry, 1 seeded little nuts enclosed in the persistent sepal. Spherical, wrinkled and brown. Not mucilaginous in water. -Calix closes when ready to seed	The plant is indigenous to tropical areas e.g. West Africa It is cultivated in Ceylon, South Sea Islands, and also within Nepal, and Bengal	Identified as Raam tulsi by South Asian communities surveyed in the UK.	G30_768 ITS AGACCGCGAACACGTGTTTAACTCATCCCCCGCCGATCGGCGTGCGGGCTAACGAACCCCGGGCGCGGAAAGCGCCAAGGAAAACTGTAGGTAGCGTCGGTCCCCCATCCGTTCGCGGCTCGTGCGGGGGATGCGGACGTCTATCGAATATCAAAACGACTCTCGGCAACGGATATCTCGGCTCTCGCATCGATCAAGAACGTAGCGAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTCTTTGAACGCAAGTTGCGCCCGAAGCCATTAGGCCGAGGGCACGTCTGCCTGGGCGTCACGCATCGCGTCGCCCCCTTCCCGGCGCACCGCGCTCGGGACGGGGGGACGGATATTGGCCTCCCGTGCGCCCCGGTGCGCGGCCGCCGGCCAAATGCGATCCCCGGCCAGGCTCCCTTCGGGGACGAGCAAGGCTCCCGGCGTCGGCAGAAGTGGTGGTTGAAACATCTCAATCTCGCGTCTCGTCGCGCTTCCGGGTCGTCCGAGCGGCTCCAAAAATAACCCAATGGTGCGGCCGTTACGCCGCGCCCTCGACCGCGGACCCCAGGTCAGG *trnH-psbA* CATCCGCCCCTCTACTATAAAAAAGTCAATTTTCTATTTATTTACGAATTCACTTTTTCAATTTAAATTTTTTTTTCAAAATTGTAAAACAAAGATAGAATTTCAATCATTTTTTTATCTTCTTGCTTTTATGGAAAGAAAAGTCTAGGTAAGTCCAATACTACTAGAAAATACAAAGGACTTCTAATTGAAAATAAAGAAAGGAGCAATAAACCCTCTTTTGATAGAACAAGAAAGGGTTTATTGCTCCTTATATTTATCTATATTTCAAAAACTTCTACACACCAAGAACAAGTCTTAGCCATTTGTTGGAGCTTCAATAGCAGCTAG

Image:	Species:	General Description:	Description of Leaves:	Description of Flowers: (Inflorescence)	Description of Fruit:	Native to:	Any other information:	DNA Sequence:
	***Ocimum kilimandscharicum***	-Perennial -An evergreen -Shrubby -Very hairy -Grows larger then tenuiflorum	-pubescent quadrangular branchlets with simple leaves that are opposite and oblong, narrow at the base and deeply serrated -Rounded leaves	-Purple flowers	-seeds are black and very small, -oval shaped -1mm	East Africa	Common names: Kilimanjaro basil, Camphor basil, Hoary basil, Fever plant	G31_676 ITS AAACCAACAAAAGTTTTACAAATGA AAACAGCCGCCCCCCCCCAGGAGAA GAAGGCGGGGCGAACAAAAAACCCC GCGGAAAAACCAACAAAAAAAAAA ACAAAAGGCGCCCCCCCCCCCCCC CCCGCCCCGGGGCGTGGGGGGGGG CGGGCCTCCATCGAAGAAAAACAAA AAAAAACTCAGCAAAAGATAACTCC GCTCTTGCATTGAAGAAAAAAGAAA AGAAAGCCAAAATGGGGTTAAAT GAAAAAAACCGTGAAACAAAAAGTT TTTGAAAACAAAATGGGCCCGAAAA CATCAGGGCCAGGGCACCTCTGCCT GGGCGTCAAGCAAAGCGTCGCCCCC CCCATCCCCGCGCACCGCGCTTGGGT AGGGGGGGGGGATACTGGCCTCCCG TGGGCCCGGCCGGCGGGGCGGCCAA AATGCATATCCCCCGGCGACCCGCG TCGCGAAAAGTGGTGGGTAAACTCA TTAATTTCCCCGT *trnH-psbA* GCCCCACTATTCTAAAAAAGTAAATT TTCTATTTATTTAGCAATTCACTTTTT GAATTTTTTTTTTCAAAATTGTAAAA CAAAAGCAAAAGATAGAATTTCAAT CATTTTTTATCTTCTTTCTTTATGG AAAGAAAAGTCTAGGTAAGTTCCAAT ACTACTAGAAAAATACAAAAGGACT TCTAATTGAAAATAAAAGAAAGGAG CAATAAACCCTCTTTTGATAGAACAA GAAAGGGTTTATTGCTCCTTATATTT ATCTATATTTCAAAACTTCTACACA CCAAGAACAAGTCTTAGCCATTTGTT GGAGCTTCAATAGCAGCTAG
	***Ocimum tenuiflorum***	- Short-lived perennial herb. - Up to 1 m tall square stem	-Green / purple -Elliptic (narrow oval) in shape -opposite decussate leaves, with numerous oil glands which secrete aromatic oils giving the plants distinct odours	-smaller flowers than *O. basilicum* -white and yellow to purple -Flowers are more spread out -can see the stalk for each flower.	Four small brown nutlets, which, unlike basil, do not produce a lot of mucilage when wet. dark brown odourless but pungent tasting seed	South Asia	Other species in the *Ocimum* genus which may be kept as tulsi include: *O. ammericanum, O. basilicum, O. canum, O. camphora, O.kilimandscharicum* and *O. micranthum* Identified as Shyam/ Krishna tulsi.	ITS_x10 reference samples AGACCGCGAACACGTGTTCAAGCGA ATCCCCCGCGTCCCCCCCGGCGG CGCGCGGGGCAACCAACCCCGGGCG CGGAACGCCCAAGGAAAACGTAAC TCGGCGCCGGCCCCCCGCATCCCGTC CGCGGGTCCTGCGCGGGGCGCGGGC GTCTCTGAATCTCAAACGACTCTC GGCAACGGATATCTCGGCTCTCGCA TCGATGAAGAACGTAGCGAAATGCG ATACTTGGTGTGAATTGCAGAATCCC GTGAACCATCGAGTCTTTGAACGCA AGTTGCGCCCGAAGCCATTAGGCCG AGGGCACGTCTGCCTGGGCGTCACG CATCGCGTCGCCCCCCTCCCCGCGCG CCGCGCTCGGGACGGGGGGCGGATG TTGGCCTCCGTCGCCCGCCGCCGC GGCCGGCCCAAACCCGATCCCCCGG CGACCCGCGTCGCGACAAGTGGTGG TTGCAACGTCTCAATCTCGCGTCTCGT CGCTCGCGGGCCGTCCGCCGCGGCT CCGAAGAGGACCCAACGGCGCGGCC AAGCCGCCGCTCCCTCGACCGCGAC CCCA *trnH-psbA*_x7 reference samples CATCCGCCCCTCTACTCTAAAAATCT AAAAAGAAAATTTTCTATTTATTTAC GAATTCACTTTTTCAATTTGAATTTTT TTTGCAAAATTGTAAAACAAAAGAT AAAATTTCAATCATTTTTTTTATCTTC TTTCTTTTATGGAAAGAAAAGTCTAG GTAAGTCCAATACTACTAGAAAAAT AGAAAAGGACTCATAATTGAAAAGA AAAGAAAGGAGCAATAAACCCTCTT TTGATAGAACAAGAAAGGTTTATT GCTCCTTATATTTATCTATATTTCAA AAACTTCTACACACCAAGAACAAGT CTTAGCCATTTGTTGGAGCTTCAATA GCAGCTAG

*Images taken from specimen grown at De Montfort University personally and with Mike Storer (Technician).

**Information from the table obtained from various sources (Maimes, 2001; WHO, 2002b; Paton et al., 2005; Joshi et al., 2012; meeting with A. Paton, 27.02.2014 at The Royal Botanic Gardens Kew).

***DNA sequences are consensus sequences of three independent reads, of samples grown from seeds, DNA extracted and a PCR with ITS and *trnH-psbA* regions.

Please refer to the external memory device for all the sample sequences

Appendix 16 Traditional Herbal Medicines Focus Group

**Please refer to the external memory device*

Appendix 16 contains:

1. Ethical amendment application form for focus groups.
2. Traditional Herbal Medicines focus group questions.
3. Focus group participant information leaflet and confirmation of attendance letter.